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Annotated List of the Leaf Beetles (Coleoptera: Chrysomelidae) of Kentucky: Subfamily Chrysomelinae

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ABSTRACT

An examination of leaf beetle specimens (Coleoptera: Chrysomelidae) in the five largest beetle collections in Kentucky, recent inventory work in state nature preserves, and a review of the literature revealed twenty-two species of the subfamily Chrysomelinae present in Kentucky, eight of which were previously unreported for the state. Distribution maps and label data are presented for the twenty-two Kentucky species of the subfamily Chrysomelinae including spatial (state and Kentucky county records), temporal (years and months of collection in Kentucky), and plant association information. The following species are reported from Kentucky for the first time: *Chrysolina cribaria* (Rogers), *Chrysolina quadrigemina* (Suffrian), *Calligrapha californica coreopsivora* Brown, *Calligrapha multipunctata* (Say), *Calligrapha pnirsa* Stål, *Calligrapha spiraeae* (Say), *Gastrophysa cyanea* F. E. Melsheimer, and *Phratora americana americana* (Schaeffer).
KEY WORDS: Kentucky, leaf beetles, Chrysomelidae, biodiversity, new state records

INTRODUCTION

This paper is the third in a series intended to present a synopsis of the historical collection data on leaf beetles (Coleoptera: Chrysomelidae) from the major Coleoptera collections in Kentucky and augment these data with new information gained from recent monitoring in state preserves and other locations. The first two papers presented information on the subfamilies Cassidinae (Barney et al. 2007) and Donaciinae and Criocerinae (Barney et al. 2008), and this paper will address the Chrysomelinae.

The subfamily Chrysomelinae is a group of leaf beetles with 135 species in 16 genera in America north of Mexico (Daccordi 1994; Riley et al. 2002). Several species in this subfamily are of economic importance, especially the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), and two species of *Chrysolina* were introduced into North America as biological control agents for Klamath weed (*Hypericum perforatum* L.): *C. hyperici hyperici* (Forster) and *C. quadrigemina* (Suffrian) (Wilcox 1972).

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The purpose of this study is to present the historical and current knowledge of the distribution, abundance, and plant associations of chrysomeline leaf beetles in Kentucky.

MATERIALS AND METHODS

To establish a historical perspective, leaf beetle specimens from the major insect collections in Kentucky (and from collections located in other states, but known to contain Kentucky specimens) were examined, reidentified, and their label data recorded. The following collections were studied: the Brigham Young University Collection (BYUC, Provo, UT); the Charles Dury Collection at the Cincinnati Museum of Natural History (CMNH, Cincinnati, OH); the private collection of Charles Wright, the Kentucky Beetles Project Collection (CWIC, Frankfort, KY), which was established in 1991 in an effort to document Coleoptera within the state; the Kentucky State University Insect Collection (KYSU, Frankfort, KY), which houses the specimens generated by the Kentucky Leaf Beetle Biodiversity Project; the private collection of Robert J. Barney (RJBC, Frankfort, KY), which comprises two time periods of collecting in Kentucky, 1976–1984 and 2004–2007; the University of Kentucky Insect Collection (UKIC, Lexington, KY), which contains the Charles V. Covell, Jr. Collection (emeritus professor of the University of Louisville); and the Western Kentucky University Collection (WKUC, Bowling Green, KY).

We currently are conducting extensive collecting in many grass-dominated barrens and rock outcrop (glade) communities that are known for possessing uncommon plants and plant associations (Jones 2005). These sites are primarily in state nature preserves that have never been surveyed for plant-feeding beetles. Most specimens were collected by the senior author within five state nature preserves in 2004–2007: Crooked Creek Barrens (Lewis County) and Blue Licks Battlefield (Robertson County) in northeastern Kentucky, Eastview Barrens (Hardin County) and Thompson Creek Glades (LaRue County) in central Kentucky, and Raymond Athey Barrens (Logan County) in western Kentucky. Voucher specimens are housed in the Kentucky State University Insect Collection.

For each chrysomeline species documented for Kentucky, the following data are presented: state-level distribution in the United States (from Riley et al. 2003), Kentucky county records, abundance by year and month in Kentucky, and specimens per collection. When present on specimen labels, other pertinent information, such as the method of collection and plant association information, is presented in the "Comments" section for each species. This information provides the opportunity to determine, from a historical perspective, abundance, seasonality, and distribution. One should note that plant collection records taken from specimen labels are notoriously inaccurate and may not reflect true host plants (Clark et al. 2004).

RESULTS

According to the "Catalog of Leaf Beetles of America North of Mexico" (Riley et al. 2003), there were 48 species of Chrysomelinae recorded in at least one of the seven states contiguous to Kentucky, thus establishing a "ballpark" estimate for the state. However, in that work only 14 species were listed from Kentucky. An examination of 846 chrysomeline leaf beetle specimens from the major collections in the state and others known to contain Kentucky specimens revealed 21 species, including 13 of the 14 recorded in Riley et al. (2003) plus eight new state records (Table 1). A twenty-second species, *Gastrophysa polygoni* (L.), was reported from Kentucky by Riley et al. (2003).

The state collection at the University of Kentucky (UKIC) contains a total of 488 chrysomeline leaf beetles representing 13 species, including four of the new state records reported herein. This collection contains the oldest in-state specimen records for Kentucky leaf beetles, with collection dates as early as 1889. The CWIC collection has 59 specimens representing ten species, including one of the new state records reported herein. The collection at WKUC has 41 specimens of seven species. Recent collecting in state nature preserves (the KYSU collection) has produced 154 specimens of eleven species and three new state records. The RJBC collection contains 61 specimens of nine species from Kentucky. An examination of the BYUC revealed 37 specimens in eleven

Table 1. List of Chrysomelinae (Coleoptera: Chrysomelidae) recorded from Kentucky, with number of Kentucky specimens examined, number of Kentucky county records, range of years of collection in Kentucky, and new state records.

Chrysolina cribaria (Rogers)	4 specimens: 3 counties, 2004–2006 (new state record)
Chrysolina quadrigemina (Suffrian)	4 specimens: 1 county, 2005 (new state record)
Calligrapha alnicola Brown	1 specimen: 1 county, 1994
Calligrapha androwi S. Clark & Cavey	1 specimen: 1 county, 1994
Calligrapha bidenticola Brown	17 specimens: 7 counties, 1894–2006
Calligrapha californica coreopsivora Brown	1 specimen: 1 county, 2006 (new state record)
Calligrapha multipunctata (Say)	1 specimen: 1 county, 1946 (new state record)
Calligrapha philadelphica (L.)	4 specimens: 2 counties, 1988–1994
Calligrapha pnirsa Stål	1 specimen: 1 county, 1948 (new state record)
Calligrapha spiraeae (Say)	2 specimens: 2 counties, 1983–1992 (new state record)
Zygogramma suturalis (F.)	111 specimens: 23 counties, 1889–2007
Labidomera clivicollis (Kirby)	64 specimens: 26 counties, 1892–2007
Leptinotarsa decemlineata (Say)	88 specimens: 10 counties, 1889–2007
Leptinotarsa juncta (Germar)	85 specimens: 31 counties, 1892–2006
Gastrophysa cyanea F. E. Melsheimer	146 specimens: 13 counties, 1890–2004 (new state record)
Gastrophysa polygoni (L.)	State-level literature record only
Phaedon viridis F. E. Melsheimer	6 specimens: 2 counties, 1891–1895
Phratora americana americana (Schaeffer)	9 specimens: 1 county, 1974 (new state record)
Chrysomela interrupta F.	4 specimens: 2 counties, 1988–1994
Chrysomela knabi knabi Brown	271 specimens: 31 counties, 1891–2006
Chrysomela scripta F.	8 specimens: 7 counties, 1901–2006
Plagiodera versicolora (Laicharting)	18 specimens: 8 counties, 1965–2006

species. Six specimens in two species of chrysomeline leaf beetles were found in the historical Dury Collection (CMNH), which comprises approximately 75,000 specimens primarily collected between 1871 and 1931 in the Cincinnati/northern Kentucky area (Vulinec and Davis 1984).

Chrysolina cribaria (Rogers) (Figure 1A) (new state record)

Kentucky Counties: Hardin, LaRue, Logan
Years: 2004 (1), 2005 (1), 2006 (2)
Months: June (2), July (1), September (1)
Abundance: 4 specimens: 4-KYSU

Comments: All four specimens were recently collected in state nature preserves: Eastview Barrens, Thompson Creek Glades, and Raymond Athey Barrens.

Chrysolina quadrigemina (Suffrian) (Figure 1B) (new state record)

Kentucky Counties: Logan
Years: 2005 (4)
Months: June (4)
Abundance: 4 specimens: 4-KYSU

Comments: All four specimens were recently collected in one month in one management unit of Raymond Athey Barrens

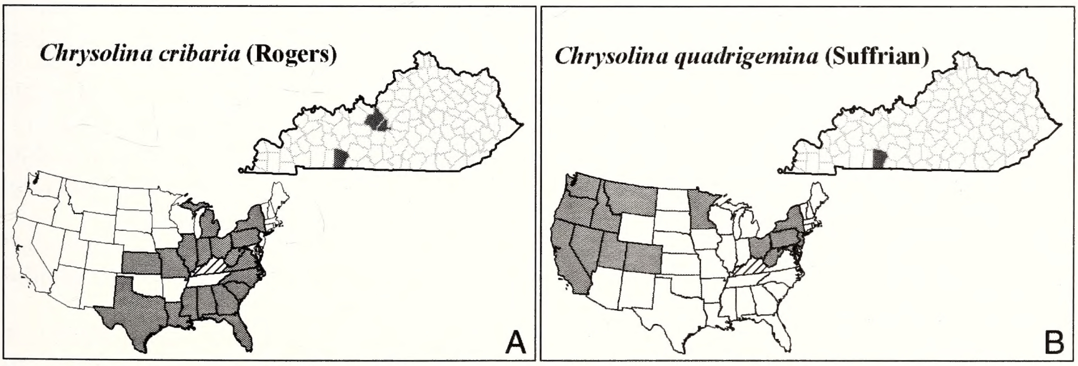

Figure 1. The known distribution of Chrysomelinae (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

State Nature Preserve. This species was introduced in eastern Canada and the Pacific Northwest to control Klamath weed and feeds on *Hypericum* spp. (Clark et al. 2004).

Calligrapha alnicola Brown (Figure 2A)

Kentucky Counties: Rowan
Years: 1994 (1)
Months: May (1)
Abundance: 1 specimen: 1-BYUC
Comments: This species feeds on *Alnus incana* (L.) Moench. (Clark et al. 2004).

Calligrapha androwi S. Clark & Cavey (Figure 2B)

Kentucky Counties: Rowan
Years: 1994 (1)
Months: May (1)
Abundance: 1 specimen: 1-California Academy of Sciences
Comments: This is a recently described species from specimens collected in Ohio, Kentucky, West Virginia and Alabama. This paratype specimen is housed in the California Academy of Sciences (Clark and Cavey 1995).

Calligrapha bidenticola Brown (Figure 2C)

Kentucky Counties: Anderson, Bracken, Fayette, Franklin, Lewis, Russell, Warren
Years: 1894 (4), 1928 (1), 1938 (1), 1992 (2), 1993 (1), 1995 (1), 1998 (2), 2005 (4), 2006 (1)
Months: April (1), May (1), June (1), July (5), August (6), September (3)
Abundance: 17 specimens: 2-BYUC, 4-CWIC, 4-KYSU, 6-UKIC, 1-WKUC
Comments: This species has been associated with Asteraceae (Clark et al. 2004).

Calligrapha californica coreopsivora Brown (Figure 2D) (new state record)

Kentucky Counties: Franklin
Years: 2006 (1)
Months: May (1)
Abundance: 1 specimen: 1-KYSU
Comments: This single specimen, a new state record, was collected in a recently established local nature preserve in Frankfort, KY.

Calligrapha multipunctata (Say) (Figure 2E) (new state record)

Kentucky Counties: Fayette
Years: 1946 (1)
Months: October (1)
Abundance: 1 specimen: 1-UKIC
Comments: This species feeds on *Salix* spp. (Clark et al. 2004).

Calligrapha philadelphica (L.) (Figure 2F)

Kentucky Counties: McCreary, Menifee
Years: 1988 (1), 1990 (1), 1994 (2)
Months: May (4)
Abundance: 4 specimens: 4-BYUC
Comments: Hosts are species of *Cornus* (Clark et al. 2004).

Calligrapha pnirsa Stål (Figure 2G) (new state record)

Kentucky Counties: Lincoln
Years: 1948 (1)
Months: April (1)
Abundance: 1 specimen: 1-UKIC
Comments: This species feeds on *Tilia americana* (L.) (Clark et al. 2004).

Calligrapha spiraeae (Say) (Figure 2H) (new state record)

Kentucky Counties: Fayette, Franklin
Years: 1983 (1), 1992 (1)
Months: April (1), June (1)
Abundance: 2 specimens: 1-CWIC, 1-RJBC
Comments: The host of this species is *Physocarpus opulifolius* (L.) Maxim. (Clark et al. 2004).

Zygogramma suturalis (F.) (Figure 3A)

Kentucky Counties: Barren, Bracken, Breathitt, Carter, Fayette, Franklin, Grayson, Hardin, Jefferson, Kenton, LaRue, Laurel, Lewis, Lincoln, Logan, Muhlenburg, Pendleton, Robertson, Rowan, Russell, Warren, Washington, Woodford
Years: 1889 (6), 1890 (2), 1891(4), 1892 (2), 1893 (1), 1894 (9), 1895 (3), 1912 (1), 1913 (2), 1917 (1), 1937 (10), 1938 (3), 1944 (3), 1948 (1), 1962 (1), 1965 (1), 1968 (4), 1970 (1), 1977 (1), 1979 (1), 1983 (2), 1984 (11), 1987 (5), 1989 (1), 1991 (1), 1994 (3), 1995 (3), 1998 (3), 2001 (3), 2004 (3), 2005 (9), 2006 (4), 2007 (6)
Months: March (1), April (3), May (11), June (17), July (36), August (33), September (7), October (3)
Abundance: 111 specimens: 6-BYUC, 3-CWIC, 19-KYSU, 21-RJBC, 52-UKIC, 10-WKUC

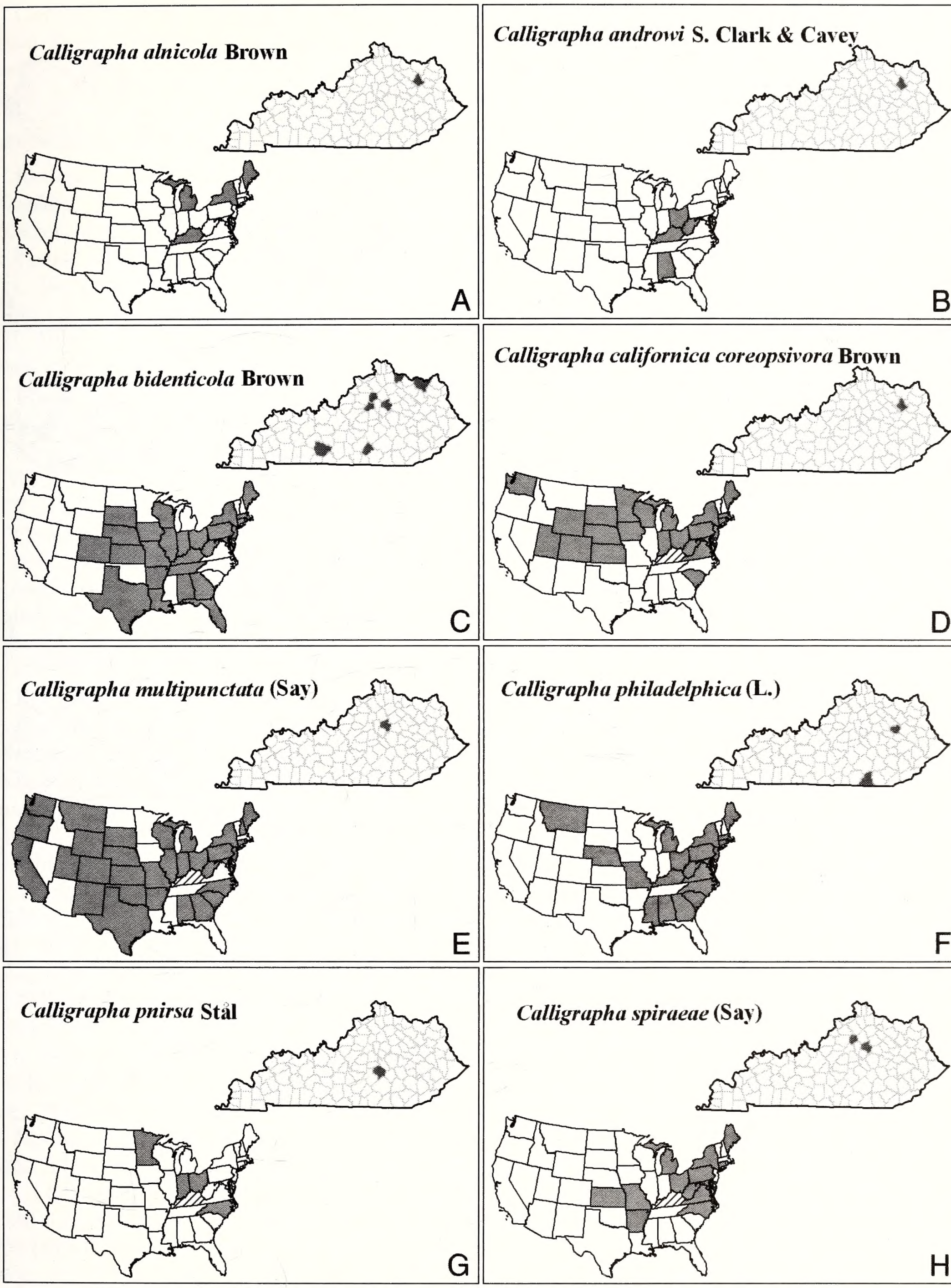

Figure 2. The known distribution of Chrysomelinae (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

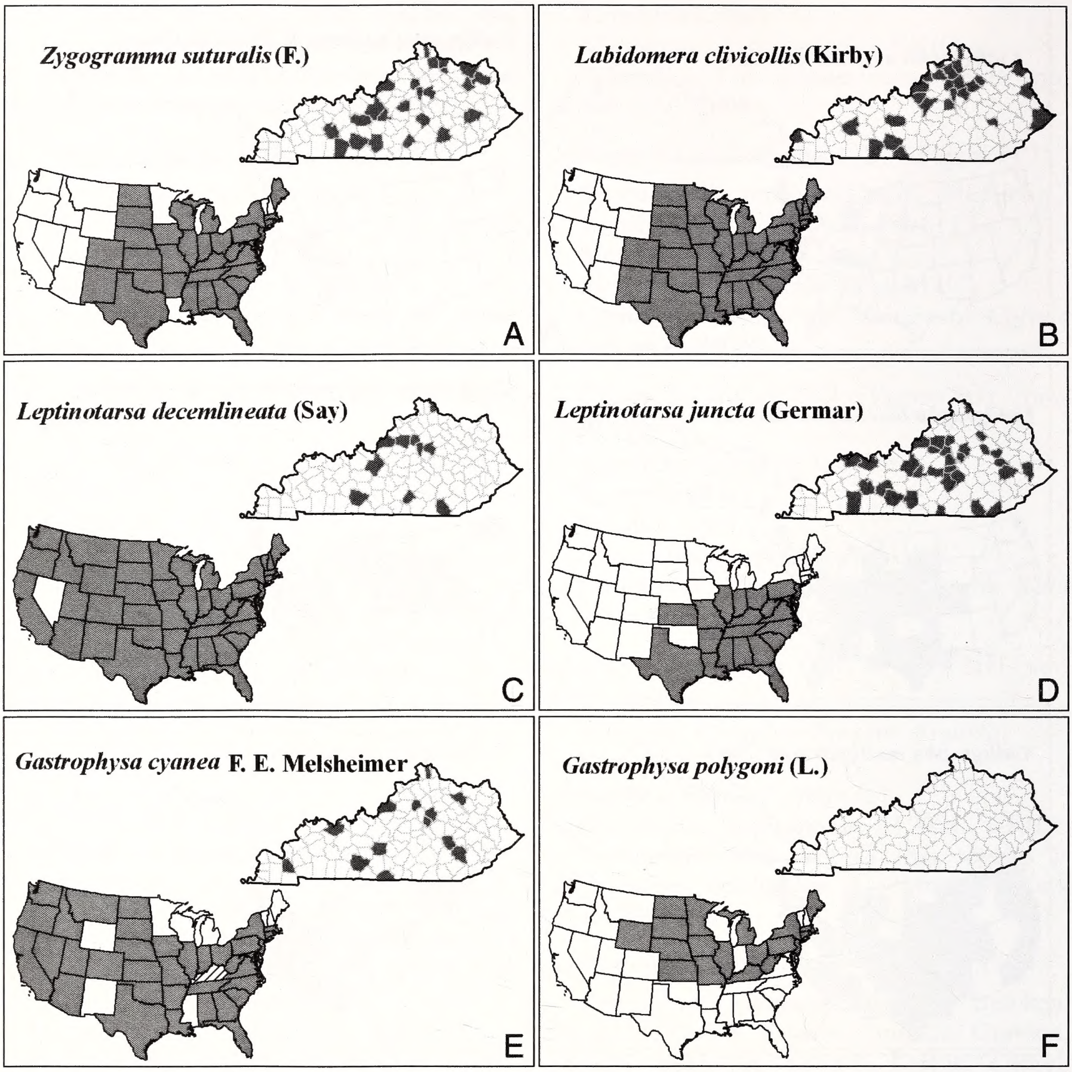

Figure 3. The known distribution of Chrysomelinae (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

Comments: Label data on UKIC specimens from the 1890s noted 'smartweed', 'A. cornnut', and 'English bluegrass'. This species feeds on ragweeds, *Ambrosia* spp. (Clark et al. 2004).

Labidomera clivicollis (Kirby) (Figure 3B)

Kentucky Counties: Allen, Ballard, Bourbon, Bullitt, Carroll, Fayette, Franklin, Grant, Grayson, Greenup, Henry, Hopkins, Jefferson, Kenton, Lawrence, Logan, Mercer, Nelson, Nicholas, Oldham, Owen, Owsley, Pike, Scott, Warren, Woodford

Years: 1892 (1), 1893 (2), 1894 (2), 1921 (1), 1938 (3), 1939 (1), 1940 (1), 1941 (2), 1944 (1), 1946 (6), 1947 (1), 1948 (3), 1950 (4), 1956 (1), 1959 (1), 1961 (1), 1965 (1), 1966 (1), 1967 (1), 1968 (1), 1969 (1), 1971 (4), 1972 (1), 1973 (2), 1978 (1), 1979 (2), 1983 (1), 1984 (1), 1987 (2), 1991 (1), 1993 (2), 1995 (1), 1999 (1), 2002 (1), 2003 (3), 2004 (1), 2005 (3), 2007 (1)

Months: April (2), May (8), June (16), July (7), August (7), September (10), October (13), November (1)

Abundance: 64 specimens: 1-BYUC, 9-CWIC, 3-KYSU, 4-RJBC, 36-UKIC, 11-WKUC

Comments: Label data on UKIC specimens from the 1890s noted '*Asclepias incarnata*', and '*Eupatorium perfoliatum*'. This species feeds on Asclepiadaceae (Clark et al. 2004).

Leptinotarsa decemlineata (Say) (Figure 3C)

Kentucky Counties: Campbell, Fayette, Franklin, Hardin, Jefferson, Russell, Shelby, Warren, Whitley, Woodford

Years: 1889 (3), 1892 (34), 1915 (4), 1922 (1), 1928 (2), 1937 (2), 1961 (1), 1965 (1), 1968 (2), 1971 (3), 1978 (1), 1983 (2), 1984 (1), 1988 (1), 1991 (1), 1998 (5), 2001 (1), 2005 (20), 2007 (3)

Months: April (1), May (10), June (12), July (56), August (7), September (1), October (1)

Abundance: 88 specimens: 5-CMNH, 1-CWIC, 23-KYSU, 3-RJBC, 52-UKIC, 4-WKUC

Comments: The Colorado potato beetle has a well documented association with *Solanum tuberosum* L. Labels noted some specimens collected by Malaise trap.

Leptinotarsa juncta (Germar) (Figure 3D)

Kentucky Counties: Anderson, Barren, Bell, Boyle, Breathitt, Butler, Campbell, Casey, Christian, Clinton, Daviess, Fayette, Floyd, Franklin, Hardin, Henderson, Jefferson, Jessamine, LaRue, Lincoln, Marion, Menifee, Mercer, Montgomery, Muhlenberg, Nicholas, Shelby, Spencer, Warren, Whitley, Wolfe

Years: 1892 (1), 1893 (1), 1905 (5), 1911 (1), 1922 (2), 1923 (1), 1925 (1), 1927 (2), 1935 (3), 1937 (9), 1938 (8), 1939 (1), 1940 (2), 1942 (1), 1944 (2), 1945 (2), 1946 (5), 1947 (2), 1948 (3), 1949 (2), 1950 (3), 1951 (3), 1952 (2), 1953 (1), 1955 (1), 1956 (1), 1957 (1), 1963 (1), 1967 (3), 1968 (1), 1983 (1), 1987 (2), 1995 (1), 2001 (1), 2003 (2), 2004 (1), 2005 (3), 2006 (3)

Months: March (3), April (8), May (24), June (12), July (5), August (8), September (17), October (7), December (1)

Abundance: 85 specimens: 1-BYUC, 1-CWIC, 1-KYSU, 8-RJBC, 70-UKIC, 4-WKUC

Comments: This species is often called the false potato beetle and feeds on *Solanum carolinense* L. (Clark et al. 2004). Jacques (1988) cites one Kentucky record for Campbell County in the Purdue University collection.

Gastrophysa cyanea F. E. Melsheimer (Figure 3E) (new state record)

Kentucky Counties: Clay, Daviess, Fayette, Fleming, Franklin, Hart, Jackson, Jefferson, Jessamine, Kenton, Marshall, Monroe, Warren

Years: 1890 (4), 1891 (69), 1892 (3), 1895 (3), 1901 (1), 1917 (8), 1923 (1), 1925 (1), 1930 (12), 1937 (4), 1938 (2), 1944 (1), 1949 (1), 1963 (1), 1968 (6), 1970 (4), 1971 (1), 1975 (5), 1976 (2), 1979 (5), 1992 (1), 1993 (1), 2000 (2), 2004 (8)

Months: January (2), March (2), April (16), May (35), June (89), August (2)

Abundance: 146 specimens: 12-CWIC, 7-RJBC, 120-UKIC, 7-WKUC

Comments: Label data (UKIC 1890s) noted collection from smartweed, lawn, wheat, dock, red clover, oats, white clover, *Rumex crispus*, and by Malaise trap. Normal hosts are species of *Rumex* (Polygonaceae) (Clark et al. 2004).

Gastrophysa polygoni (L.) (Figure 3F)

Comments: No specimens from Kentucky were seen during this study, but it was listed from the state by Riley et al. (2003). This species feeds on Polygonaceae (Clark et al. 2004).

Phaedon viridis F. E. Melsheimer (Figure 4A)

Kentucky Counties: Ballard, Fayette

Years: 1891 (1), 1894 (4), 1895 (1)

Months: May (1), July (1), November (1), December (3)

Abundance: 6 specimens: 6-UKIC

Comments: Label data (UKIC 1890s) noted collection from alfalfa, grain, clover, and weeds. The Ballard County (Wickliffe) record is from Balsbaugh's (1983) revision of *Phaedon*. Hosts of this species are Brassicaceae (Clark et al. 2004).

Phratora americana americana (Schaeffer) (Figure 4B) (new state record)

Kentucky Counties: Nelson

Years: 1974 (9)

Months: August (9)

Abundance: 9 specimens: 1-RJBC, 8-UKIC

Comments: This species is associated with *Salix* (Salicaceae) (Clark et al. 2004).

Chrysomela interrupta F. (Figure 4C)

Kentucky Counties: Jackson, Whitley

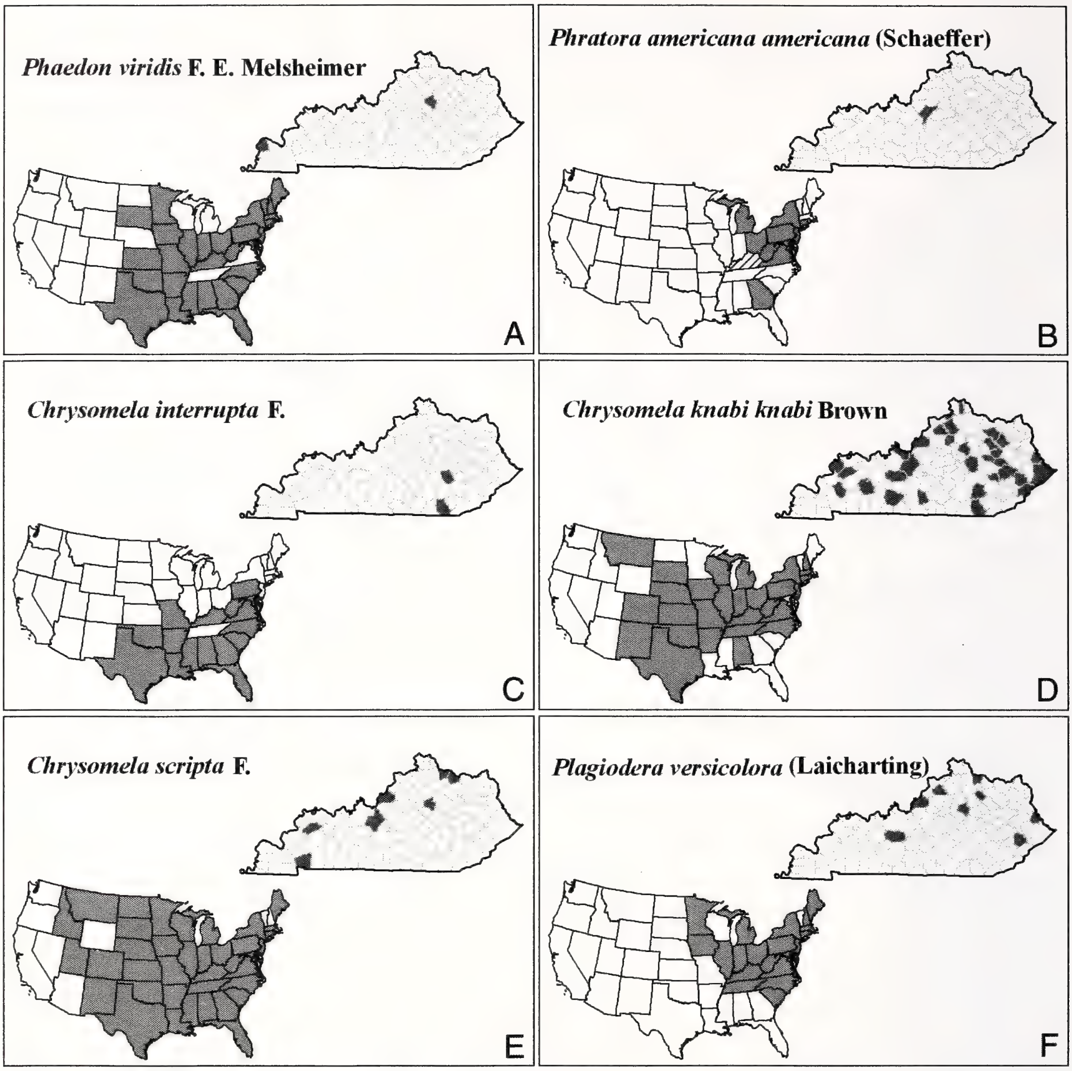

Figure 4. The known distribution of Chrysomelinae (Coleoptera: Chrysomelidae) illustrated in grey shading for Kentucky counties and states of the United States. New state records reported herein are shown in cross-hatch.

Years: 1988 (2), 1994 (2)

Months: May (4)

Abundance: 4 specimens: 4-BYUC

Comments: Both adults and larvae are associated with *Alnus* (Betulaceae) (Clark et al. 2004).

Chrysomela knabi knabi Brown (Figure 4D)

Kentucky Counties: Bath, Breathitt, Caldwell, Fayette, Fleming, Franklin, Gallatin, Grayson, Hardin, Henry, Jefferson, Kenton, Knott, Laurel, Letcher, Madison, Martin, Meade, Menifee, Metcalfe, Muhlenberg, Owen, Owsley, Pike, Powell, Rowan, Union, Warren, Webster, Whitley, Wolfe

Years: 1891 (4), 1895 (1), 1901 (12), 1927 (3), 1937 (1), 1939 (10), 1940 (2), 1941 (3), 1942 (10), 1958 (7), 1965 (1), 1966 (1), 1970 (58), 1971 (6), 1980 (11), 1982 (5), 1983 (1), 1990 (1), 1992 (2), 1993 (1), 1994 (17), 1995 (2), 2001 (1), 2003 (5), 2004 (4), 2005 (87), 2006 (14)

Months: April (146), May (88), June (26), July (9), October (1)

Abundance: 271 specimens: 12-BYUC, 1-CMNH, 25-CWIC, 93-KYSU, 5-RJBC, 131-UKIC, 4-WKUC

Comments: There is no county, year or month available for one CMNH specimen. This species is associated with *Salix* (Salicaceae) (Clark et al. 2004).

Chrysomela scripta F. (Figure 4E)

Kentucky Counties: Bracken, Fayette, Hardin, Jefferson, Mason, Trigg, Webster

Years: 1901 (1), 1970 (1), 1971 (1), 1998 (3), 2005 (1), 2006 (1)

Months: May (2), June (2), July (4)

Abundance: 8 specimens: 3-BYUC, 1-CWIC, 1-KYSU, 3-UKIC

Comments: The Mason County (Maysville) record is from Brown's (1956) revision of *Chrysomela*. This species is associated with *Salix* and *Populus* (Salicaceae) (Clark et al. 2004).

Plagiodera versicolora (Laicharting) (Figure 4F)

Kentucky Counties: Bracken, Fayette, Grayson, Henry, Jefferson, Knott, Martin, Nicholas

Years: 1965 (1), 1981 (7), 1983 (4), 1998 (2), 2003 (2), 2006 (1)

Months: May (8), June (6), July (2), August (1)

Abundance: 18 specimens: 2-BYUC, 2-CWIC, 1-KYSU, 11-RJBC, 2-UKIC

Comments: There is no county, year or month available for one UKIC specimen. This species is associated with Salicaceae (Clark et al. 2004).

DISCUSSION

We believe the data presented here are the most complete representation of the chrysomeline leaf beetles known from Kentucky. The large number of new state records documented here (8 of 22, or 35%) reflects a historical lack of leaf beetle collecting in Kentucky. Three of the eight new state records are based on a single specimen, and eleven of the 22 species have been documented from only one or two counties. Six species accounted for 765 of the 846 specimens reviewed in this study, leaving only 81 beetles for the other 16 species. These data, plus the fact that the ballpark estimate for Kentucky Chrysomelinae is 48 species and that we found only 22 species, indicate that many more species may still out there to be found.

The host plant switch of the Colorado potato beetle, from the native buffalo bur (*Solanum rostratum* Dunal) to cultivated potato (*S. tuberosum* L.), enabled this large showy beetle, first found in the area of the Rocky Mountains, to exploit a changing landscape and become a widespread economic pest (Jacques 1988). Examples such as this are a reminder why it is so important to document the presence and distribution of our native and exotic flora and fauna.

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Diversity, Substrata Divisions and Biogeographical Affinities of Land Snails at Bad Branch State Nature Preserve, Letcher County, Kentucky

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ABSTRACT

The results of this study established that Bad Branch State Nature Preserve has at least 64 native land snail species occurring within its boundaries, representing 14 families and 35 genera. Twenty-five of the snail taxa documented at Bad Branch were new records for Letcher County; 19 species or 29% of the new records accounted for snails under 5 mm. In total, micro snail species (<5 mm) represented 45% or 28 species of the documented fauna. Two species, *Paravitrea lamellidens* Pilsbry and *Paravitrea dentilla* Hubricht were previously unknown from Kentucky. Stress in the non-metric multidimensional scaling analysis reached an acceptable level (0.118) with a three-dimensional solution. The ordination of the 19 sites indicated strong differences in the species composition of the land snail fauna between limestone and sandstone substrates. Approximately half of the species were restricted to just one substrate, with 14 restricted to sandstone and 19 restricted to limestone. Abundance (individuals/site), species richness (species/site), and percent species in the total sample were two times to almost four times greater on limestone substrate than sandstone ($P \leq 0.001$). This suggested that more sandstone habitat is required to sustain the same number of snail species as is limestone habitat and that land snail diversity in the sandstone regions of Bad Branch are working at a much larger ecological scale than in limestone regions. A number of terrestrial gastropods found in Kentucky's Pine Mountain region, in particular Bad Branch, are biogeographically significant, as they represent primary associations with the Great Smoky Mountains-Blue Ridge sections of the Cumberland Province or the Blue Ridge region of West Virginia and Virginia. These interesting snail associations are further supported by the discovery of *P. lamellidens* and *P. dentilla* at Bad Branch State Nature Preserve.

KEY WORDS: Bad Branch, land snails, Kentucky, *Paravitrea lamellidens*, *Paravitrea dentilla*

INTRODUCTION

Bad Branch State Nature Preserve is an outstanding illustration of an undomesticated environment, occurring along the crest of Pine Mountain, in Letcher County, Kentucky (Figure 1). The preserve safeguards one of the state's finest upper elevation mountain stream ecosystems, and, because of its relatively unspoiled landscape and pristine waters, Bad Branch was designated a Kentucky Wild River System in 1989 (Figure 2). The altitude, range of soil types and the preserve's physiographic location has created a wealth of biodiversity. With few exceptions, the flora and fauna remains intact. Several taxa assemblages found in these primordial mountains, an example being the vegetation, reach exceptional diversity at Bad Branch. The preserve was predicted to also have an analogous variety of land snails as well.

To better understand the richness, distribution and biogeographical affinities of land snails of the southeastern mountains of Kentucky and in particular Bad Branch Nature Preserve, an inventory was initiated in 2006. While the number of snail species occurring in the state has been well documented, approximately 170 taxa (Hubricht 1968, 1985; Branson 1973; Branson and Batch 1968, 1970, 1988), the distribution patterns and ecology of land snails remains incomplete, especially in the southeastern mountains. Recent studies in Kentucky have shown, for example, that where there are comprehensive snail inventories with an emphasis on diminutive snail taxa (<5 mm), numerous county records are added; typically more than 20

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Figure 1. Bad Branch State Nature Preserve is an outstanding illustration of an undomesticated environment, occurring along the great dividing crest of Pine Mountain, in Letcher County, Kentucky. The lower elevation Cumberland Plateau region of eastern Kentucky can be seen in the background.

species (Dourson and Feeman 2006; Dourson 2007). Small snails (<5 mm) can represent more than 40% of the snail fauna (Hubricht 1968; Hotopp 1999; Dourson 2007). The lack of species accounts in Kentucky, particularly the micro snail taxa, is also well-illustrated in Hubricht (1985) distribution records for the eastern land snails of North America that show numerous county gaps. If an inventory method fails to collect the leaf litter samples where the majority of snails (<5 mm) frequently reside, micro snail species may go unreported. Omitting micro snails from past collections has likely resulted in the vast majority of county gaps. This has resulted in many states listing a number of their terrestrial gastropods as rare or uncommon occurrences.

Site specific land snail richness and shell abundance have long been associated with a variety of geological and ecological factors. For example, terrestrial gastropods living around carbonate cliffs can exhibit large and diverse populations (Nekola 1999) but show significant declines in as little as 50 m from a calcareous source (Kalisz and Powell 2003) or limestone cliffline (Dourson 2007). Additional factors such as gradient, elevation, vegetation, litter moisture and soil cations (particularly calcium) can significantly affect land snail abundance, both in terms of richness of species and numbers of shells (Boycott 1934; Burch 1955, 1956; Atkins 1966; Agocsy 1968; Valovirta 1968; Wareborn 1970; Getz 1974; Pollard 1975; Petranka 1982; Hotopp 2002).

Less well-known is how large geophysical landscape edges might serve in bridging distinctive regions and their allied terrestrial gastropod communities. Neighboring land masses may in effect be the driving force behind the distribution and mixing of some snail faunas, acting as travel corridors for dispersal. The end result can be remarkably high land snail diversity in comparatively small

Figure 2. Bad Branch is one of the finest mountain stream ecosystems to be found in Kentucky and was designated a state wild river system in 1989. Bad Branch falls is in the background.

places. The Central Knobstone Escarpment in Powell County, Kentucky, is an example. This area forms a large geophysical landscape edge on Furnace Mountain where the Cumberland Plateau, the Knobs, and the Outer Bluegrass regions of Kentucky converge. The merging of these distinct regions was shown to harbor an exceptional number of land snail taxa, a reported 61 species co-existing within a 2 ha mesic hillside (Dourson 2007). A number of the snails documented at Furnace Mountain were established beyond their reported eastern and western limits in the state (Branson and Batch 1968; Branson 1973; Hubricht 1985).

The snail inventory at Bad Branch also was expected to further the understanding as to the extent of possibly unique biogeography assemblages of terrestrial gastropods in the southeastern mountains of Kentucky. Branson and Batch (1968, 1988) reported that the principal land snail affinity of this region of Kentucky was with the Great Smoky Mountains-Blue Ridge regions of the Cumberland Province, with some segments of the fauna showing relationships with more easterly areas, i.e., through Virginia and West Virginia. This view was further supported by Hubricht et al. (1983), whose land snail collections showed further evidence of the region's faunal affinities to the Ridge and Valley and Blue Ridge Physiographic Provinces.

STUDY AREA

Woods et al. (2002) placed the study area (Figure 3) within the Cumberland Mountain Thrust Block Ecoregion. The area is characterized by steep ridges, hills, coves, narrow valleys, and the Pine Mountain Overthrust Fault. Forest composition is highly variable and is determined by aspect, slope position, historic usage, and degree of topographical shading. Many streams in this ecoregion are cool and high gradient; with a substrate commonly consisting of cobble and boulder (riffles are common). The underlying geology

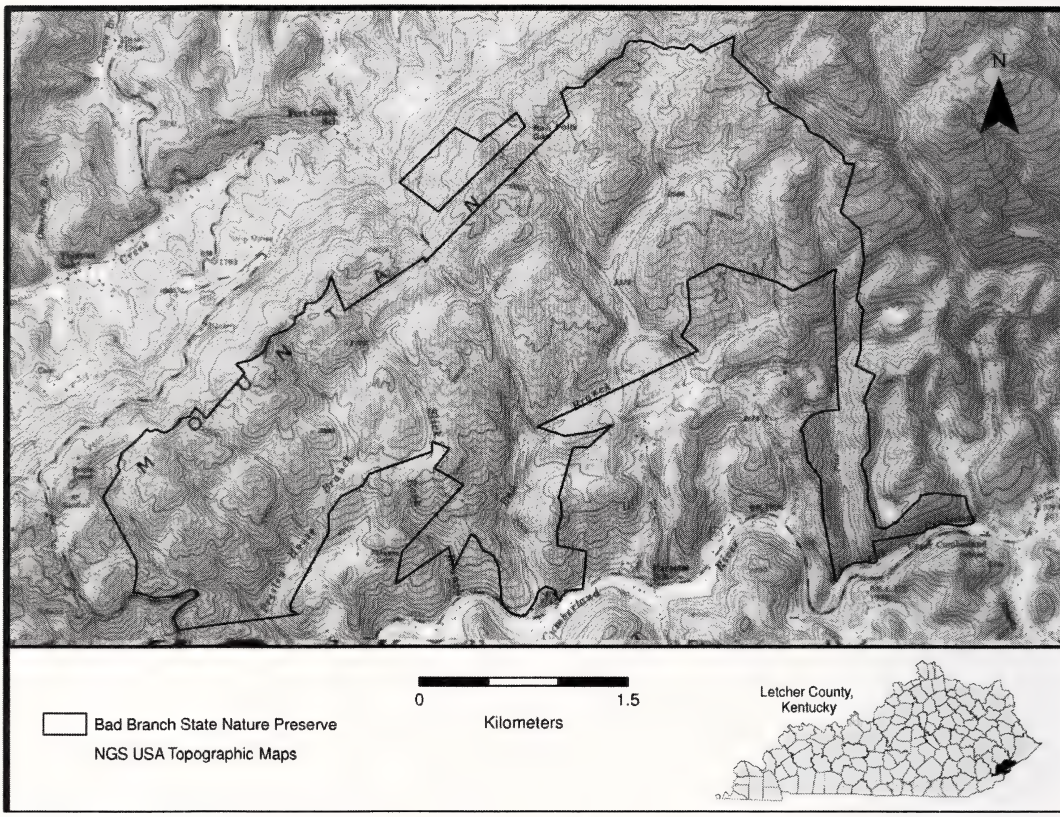

Figure 3. Map showing land snail sampling locations at Bad Branch State Nature Preserve in eastern Kentucky.

consists of Pennsylvanian shale, siltstone, sandstone, conglomerate and coal. The presence of coal mining and acidic mine drainage, as well as logging, has led to the degradation of many streams. Nutrient levels in streams are low, a result of low population density in the area, limited farming, and non-carbonate rocks.

In particular, the study area is located on Pine Mountain, which in Kentucky follows a northeast to southwest path, stretching 177 km from Breaks Interstate Park to the Kentucky-Tennessee border. Much of the mountain is the geographic border between Kentucky and Virginia. The northwest facing Monclinal Mountain consists of a steep overthrust fault, exposing chunks of limestone bedrock and scattered limestone screes. In contrast, the southeast face of Pine Mountain is gentler, exposing only the Pennsylvanian sandstones, shale and siltstones. The northern slope was significant for our current survey because it provided the necessary carbonate soils to anchor calciphile land snail species not found on the acidic southeast slopes of Bad Branch.

Pine Mountain forests are variable, depending on soils and slope exposure (Braun 1935). Both of these variables are based upon geological structure, as the mountain is a monoclinal ridge of strongly dipping strata. Braun (1935) considered four main communities on Pine Mountain; the southeast slope, ravines of the southeast slope, the summit, and the northwest slope. As with land snails, plant communities vary greatly as one proceeds across these communities. Mature soils on the mountain support mixed mesophytic forests while immature soils support a community more dominated by pine, oak, and pine-oak groups. In the mixed mesophytic forest sections, sugar maple *Acer saccharum* Marshall, basswood *Tilia americana* L., and yellow buckeye *Aescylus flava* Solander are considered to be indicator species (Braun 1950). In addition, American beech *Fagus*

grandifolia Ehrhart, cucumber magnolia *Magnolia acuminate* L., northern red oak *Quercus rubra* L., tuliptree *Liriodendron tulipifera* L., and white ash *Fraxinus americana* L. also are characteristic (Jones 2005).

Historically the upper slopes and ridgetops of this region were dominated by oak *Quercus sp.* and American chestnut *Castanea dentata* (Marshall). However, the American chestnut component of the forest was decimated by blight during the early to mid 19th century. Pine species may also be prevalent on some ridges. Especially ubiquitous is Virginia pine *Pinus virginiana* Miller and shortleaf pine *P. echinata* Miller. Ravines in this area are typically dominated by eastern hemlock *Tsuga canadensis* (L.), with an understory of rhododendron *Rhododendron* species. The forests in this region are considered to be the most biologically diverse of any in the United States (Jones 2005).

METHODS

The land snail compilation of Bad Branch included collections on both the northwest and southeast slopes of Pine Mountain, and we attempted to survey in as many common to uncommon habitat types as we could find in Bad Branch State Nature Preserve. To best accomplish this task, nineteen sites (each around 0.25 ha) were established, including 5 limestone sites located on the steep north slope of Pine Mountain and 14 sandstone sites located on the more gentle south slopes of the last-named mountain. Fewer samples were taken from the limestone substrata, because it represented only about 41 ha or 3.8% of the total1069 ha that comprises Bad Branch State Nature Preserve.

A stratified random sampling method was used for site selection, a technique that focused largely on finding as many variations in both common habitat (e.g., leaf litter) to uncommon habitats or microhabitats (e.g., seeps, tree crotches, fallen and rotting hardwood trees in advanced stages of decay, depressions of moist leaf litter and or screes) occurring across the preserve. Shells of larger snail species (>5 mm) were hand-picked from under leaf litter, rocks, and logs. When outcrops of rock were encountered, snails were gleaned from the surfaces or from under small overhanging ledges where shells often accumulate. Slugs were collected from under the exfoliating bark of standing dead trees and rotting hardwood logs in advanced stages of decay.

To best represent the micro-snails (<5 mm) of Bad Branch, 19 cm^2 stratified random soil/leaf samples were also collected, each of these occurring within one of the 19 sites. The samples were thoroughly dried and then sifted through a series of sieves ranging from 4.76–0.50 mm. Snails were removed and sorted. All recovered and identifiable shells were assigned to species using Burch (1962), Pilsbry (1940, 1946, 1948), the primary author's reference collections, and various recent publications. Taxonomy follows Turgeon et al. (1998). New county records were determined using Branson (1973), Hubricht (1985) and Branson and Batch (1988).

The species composition was analyzed with non-metric multidimensional scaling (NMDS). NMDS is an ordination method that uses an iterative approach for finding the best position of n objects in a k-dimensional space. An $n \times n$ distance matrix is calculated from a site (p) $\times$ species (s) matrix, so the ordination can be based on either position of sites or species. Because it uses rank-distances, it avoids the assumption of linear relationships among variables (Clarke 1993; McCune and Grace 2002). Site $\times$ species matrices are often very sparse (i.e., a large number of cells contain zeroes), and, as a result, assumptions of linearity are inappropriate. Therefore, NMDS is often considered the best technique for unconstrained ordination of species or sites (McCune and Grace 2002). The distance matrix was based on the Jaccard index (J) of dissimilarity ($1 - J$), and the ordination was conducted on the distances between sites. The starting configuration was based on principal components analysis to reduce the possibility of the solution converging on a local rather than global minimum. The maximum number of iterations for convergence was set at 200. Plots of the distance in multidimensional space against similarity (Shephard's diagrams) were used to evaluate stress in the ordination. The number of dimensions in the final solution was determined by a plot of stress against the number of dimensions ($6 \leq k \leq 2$) and a stress threshold <0.15 (Clarke 1993).

Differences in diversity and abundance of the snail fauna between limestone and sandstone substrates were evaluated with one-way ANOVA. Separate ANOVA's were conducted for each of three dependent variables; the number of individuals per site, number of species per site, and percentage of the total snail fauna collected per site. The dependent variables were standardized by site because of the highly uneven number of samples between the two substrates. Normal probability plots, histograms, and plots of studentized residuals vs. estimated values were used to evaluate whether assumptions of linearity, homoscedasticity, and normality were met. The assumptions were met in all three analyses. One outlier (5.14 standard deviation units) was detected in the analysis of individuals therefore two analyses were conducted for individuals per site; one with and one without the outlier. The results of the analyses were similar, so we retained the record for the final analysis.

RESULTS

We collected 825 land snail specimens (454 shells from limestone and 371 shells from sandstone) representing 14 families, 35 genera, and 64 species (Table 1). Of these, 25 taxa had not been reported from Letcher County by Branson (1973), Hubricht (1985) or Branson and Batch (1988). Twenty-eight snail species or 45% of the fauna were species under 5 mm. The highest species diversity for a single site came from the north side of Pine Mountain in limestone substrata that harbored 28 land snail species, while the lowest yielding site occurred in sandstone with only 6 species represented. No snails listed as endangered, threatened, or special concern by the Kentucky State Nature Preserves Commission (2005) were revealed during the survey.

Stress in the non-metric multidimensional scaling analysis reached an acceptable level (0.118) with a three-dimensional solution. The ordination of the 19 sites indicated strong differences in the species composition of the land snail fauna between limestone and sandstone substrates (Figure 4). Approximately half of the species were found in one substrate, with 15 occurring in sandstone and 21 occurring in limestone. Seven of the terrestrial gastropods found in the limestone, *Glyphyalinia cumberlandiana* (Clapp), *Hendersonia occulta* (Say), *Pomatiopis lapidaria* (Say), *Gastrocopta contracta* (Say), *Gastrocopta pentodon* (Say), *Gastrocopta corticaria* (Say) and *Strobilops labyrinthica* (Say) are reported to be calciphile species (Hubricht 1985). Twenty-eight species were less discriminatory however, occurring on both sides of Pine Mountain usually in similar situations, under leaf litter, rocks, logs or the loose bark of dead trees in advance stages of decomposition. The ordination of the 14 sandstone sites also indicated strong differences in the species composition of the land snail fauna found within the sandstone substrata.

Abundance (individuals/site), species richness (species/site), and percent species in the total sample were 2 times to almost 4 times greater on limestone substrate than sandstone (Figure 5; $P \leq 0.001$). This suggested that more sandstone habitat was required to sustain the same number of snail species as limestone and that land snail diversity in the sandstone regions of Bad Branch were working at a much larger ecological scale than in the limestone.

Species of Special Interest

Approximately half of the snail fauna at Bad Branch were considered wide ranging species; the remaining terrestrial gastropods found have biogeographical affinities to other physiographic provinces. Land snail assemblages at Bad Branch that had associations with the Great Smoky Mountains were *Paravitrea lamellidens*, *P. placentula* Shuttleworth, *Ventridens lawae* (W. G. Binney), *P. subtilis* Hubricht, *V. collisella* (Pilsbry), *V. theloides* (Walker and Pilsbry), *Anguispira mordax* Shuttleworth (Figure 6), *Mesodon normalis* (Pilsbry), *Carychium clappi* Hubricht, *Cionella morseana* (Doherty), and *Glyphyalinia caroliniensis* (Cockerell). Species that were found to be largely associated with the Blue Ridge, specifically the states of Virginia and West Virginia included *Philomycus venustus* Hubricht, *P. flexuolaris* Rafinesque, *Paravitrea multidentata* (A. Binney), *Paravitrea dentilla*, *Stenotrema hirsutum* (Say), *Neohelix albolabris* (Say), *Glyphyalinia cumberlandiana*, *Triodopsis anteridon* Pilsbry and *Hendersonia occulta*. Finally, a measure of the

snails from Bad Branch survey including *Vertigo gouldi* (A. Binney), *Euconulus fulvus* (Müller) a Holarctic species, *Mesomphix inornatus* (Say), *M. cupreus* (Rafinesque), *Euchemotrema fraternum* (Say), *Striatura ferrea* Morse, *Pallifera dorsalis* (A. Binney), *Allogona profunda* (Say), and *Appalachina sayana* (Pilsbry) had their affinities with more northerly faunas (Branson and Batch 1968, 1988; Hubricht 1985).

Paravitrea lamellidens (new state record) was an interesting addition to Bad Branch for several reasons. Hubricht (1985) showed this snail's range to center more or less around the Great Smoky Mountains and its habitat to include pockets of deep, moist leaf litter on wooded hillsides of lower to higher elevations forests. It also has a strong affinity to rock talus. The discovery of this species at Bad Branch is a range extension of more than a hundred miles to the north (Hubricht 1985). Specimens were secured from two sites, one from rock talus and another from under moist leaf litter, at elevations of 489 m to 712 m respectively. Both sites occurred in the sandstone bedrock associated habitats. Although Branson and Batch (1968, 1988), Petranka (1982), Hubricht (1983) and others conducted concentrated site investigations of Kentucky's southeastern mountains, mostly in the counties of Bell, Harlan and western Letcher, the species was not found. This suggests that the range of *P. lamellidens* in Kentucky may be more easterly along Pine Mountain, including eastern Letcher County and perhaps Pike County as well. These two counties are among the least sampled in southeastern Kentucky. This was well illustrated by Hubricht (1985) distribution records that showed many gaps for common snail species in these two counties. Bad Branch alone produced 23 new records for Letcher County, further illustrating this information deficit. The thought that *P. lamellidens* occurs more easterly in Kentucky is based on the fact that the species was not found in Bell and Harden Counties despite intensive surveys and a record for *P. lamellidens* exists less than 50 miles to the east in Craig County, Virginia, in the Jefferson National Forest (Dourson, unpublished data).

Paravitrea dentilla (new state record) is another unexpected snail to Kentucky's land snail fauna. Hubricht (1985) showed the species only from Washington County, Virginia, and McDowell County, West Virginia. It is found in leaf litter on river bluffs (Hubricht 1985). At Bad Branch, *P. dentilla* was discovered at only one site in moist leaf litter around limestone rock talus occurring on the north slope of Pine Mountain. Four specimens were retrieved during our survey suggesting its overall rarity at Bad Branch. Given that it has not been reported by other collectors from the southeastern mountains of Kentucky, it likely represents one of the state's rarer snails.

Two other species of snails found during the survey deserving particular mention include *Vertigo parvula* Sterkii and *Striatura ferrea*. *Vertigo parvula* was first reported in Kentucky by Branson and Batch (1988). They collected one specimen from a moist wooded hillside, 7.3 km north of Richmond on SR 60 (Tates Creek Road), Madison County, Kentucky. Although their record from Madison County seems an unlikely site for the species, there are in fact other disconnected populations reported. The primary distribution of *V. parvula* lies along the Appalachian divide in Virginia, with one relatively isolated location reported from Carter County, Tennessee, and one exceptionally isolated population occurring in Summit County in northeastern Ohio (Hubricht 1985). In our study, *V. parvula* was found from two sites (a total of three specimens) located on the north side of Pine Mountain under leaf litter, at the base of limestone outcrops. The species was scarcely detected by leaf litter sampling. It is a cryptic shell of diminutive size (1.5 mm) remaining well concealed among debris. *Vertigo parvula* had fewer shells collected than any other species, suggesting that it is one of the rarer snails of Bad Branch or that the collecting methods or locations we used were inadequate at capturing this species. According to Pilsbry (1948), this is a relatively rare snail.

Harlan is the only county in Kentucky previously reported to have *Striatura ferrea* (Pilsbry 1946; Petranka 1982; Hubricht 1985). Even though Branson and Batch (1988) conducted an extensive survey of Kentucky, sampling terrestrial gastropods from 55 counties, documenting 19 families, 45 genera and 138 species, they did not find *S. ferrea*. The

Table 1. Land snail species documented at Bad Branch Nature Preserve, Letcher County, Kentucky. The ranking system under status are by the authors and do not reflect official state ratings. Keys to table: Status: KR= Kentucky Rare, LR=Limited Range in Kentucky, CO=Common; Record: SR=State Record, CR=County Record; Affinities: GSM=Great Smoky Mountains, VWV=West Virginia/Virginia, NOR=Northern, CKY= Central Kentucky, WID=Widespread in eastern North America.

Species	Substrata	Habitat	Status	Record	Affinities
HELICINIDAE					
Hendersonia occulata (Say, 1831)	Limestone	Leaf litter	LR		VWV
POMATIOPSIDAE					
Pomatiopsis lapidaria (Say, 1817)	Both	Moist litter	CO		WID
CARYCHIIDAE					
Carychium clappi Hubricht, 1959	Both	Moist litter	CO		GSM
Carychium exile H. C. Lea	Both	Moist litter	CO		WID
Carychium nannodes Clapp, 1905	Limestone	Moist litter	CO		WID
COCHLICOPIDAE					
Cochlicopa morseana (Doherty, 1878)	Both	Leaf litter	CO		GSM
PUPILLIDAE					
Columella simplex (Gould, 1821)	Limestone	Leaf litter	CO		WID
Gastrocopta contracta (Say, 1822)	Limestone	Leaf litter	CO	CR	WID
Gastrocopta corticaria (Say, 1816)	Limestone	Leaf litter	CO	CR	WID
Gastrocopta pentadon (Say, 1821)	Both	Leaf litter	CO	CR	WID
Vertigo gouldi (A. Binney, 1823)	Both	Leaf litter	CO		NOR
Vertigo parvula Sterkii, 1890	Limestone	Leaf litter	KR	CR	VWV
STROBILOPSIDAE					
Strobilops labrythinca (Say, 1817)	Limestone	Leaf litter	CO	CR	WID
HAPLOTREMATIDAE					
Haplotrema concavum (Say, 1821)	Limestone	Leaf litter	CO		WID
PUNCTIDAE					
Punctum minutissimum (I. Lea, 1841)	Both	Seeps	CO	CR	WID
Punctum vitreum H. B. Baker, 1930	Limestone	Moist litter	KR	CR	WID
HELICODISCIDAE					
Helicodiscus notius Hubricht, 1962	Limestone	Leaf litter	CO	CR	WID
DISCIDAE					
Anguispira mordax (Shuttleworth, 1852)	Limestone	Leaf litter	CO	CR	GSM
Discus patulus (Deshayes, 1830)	Both	Rotting wood	CO		WID
PHILOMYCIDAE					
Pallifera dorsalis (A. Binney, 1842)	Limestone	Moist talus	CO	CR	NOR
Philomycus flexuolaris Rafinesque, 1820	Both	Under bark	CO		VWV
Philomycus venustus Hubricht, 1953	Sandstone	Under bark	LR		VWV
HELICARIONIDAE					
Euconulus dentatus (Sterkii 1893)	Limestone	Leaf litter	CO	CR	WID
Euconulus fulvus Müller, 1774	Limestone	Leaf litter	CO		NOR
Guppya sterkii (Dall, 1888)	Both	Leaf litter	CO		WID
ZONITIDAE					
Mesomphix cupreus (Rafinesque, 1831)	Both	Leaf litter	CO		NOR
Mesomphix inornatus (Say, 1821)	Both	Leaf litter	CO		NOR
Mesomphix perlaevis (Pilsbry, 1900)	Both	Leaf litter	CO		WID
Hawaiia miniscula (A. Binney, 1840)	Limestone	Leaf litter	CO	CR	WID
Paravitrea dentilla Hubricht, 1978	Limestone	Rock structure	KR, LR	SR	VWV
Paravitrea lamellidens (Pilsbry, 1898)	Sandstone	Rock talus	KR, LR	SR	GSM
Paravitrea multidentata (A. Binney, 1840)	Both	Seeps	CO		VWV
Paravitrea placentula (Shuttleworth, 1852)	Both	Leaf litter	LR		GSM
Paravitrea subtilis Hubricht 1978	Sandstone	Leaf litter	KR, LR	CR	GSM
Glyphyalinia indentata (Say, 1823)	Limestone	Leaf litter	CO		WID
Glyphyalinia cumberlandiana (Clapp, 1919)	Both	Rock talus	CO		VWV
Glyphyalinia wheatleyi (Bland, 1883)	Sandstone	Leaf litter	CO	CR	WID
Glyphyalinia caroliniensis (Cockerell, 1890)	Sandstone	Leaf litter	CO	CR	GSM
Gastrodonta interna (Say, 1822)	Limestone	Rotting wood	CO		WID
Striatura meridionalis (Pilsbry & Feriss, 1906)	Both	Moist litter	CO	CR	WID
Striatura ferrea Morse, 1864	Sandstone	Seeps	KR, LR	CR	NOR
Ventridens demissus (A. Binney, 1843)	Sandstone	Leaf litter	CO		WID
Ventridens collisella (Pilsbry, 1896)	Sandstone	Leaf litter	LR	CR	GSM
Ventridens theloides (Walker & Pilsbry, 1902)	Both	Leaf litter	LR	CR	GSM

Table 1. Continued.

Species	Substrata	Habitat	Status	Record	Affinities
Ventridens lawae (W. G. Binney, 1892)	Both	Leaf litter	LR	CR	GSM
Ventridens ligera (Say, 1821)	Sandstone	Leaf litter	CO	CR	WID
Zonitoides arboreus (Say, 1816)	Sandstone	Under bark	CO		WID
POLYGYRIDAE					
Allogona profunda (Say, 1821)	Limestone	Leaf litter	CO		NOR
Euchemotrema fraternum (Say, 1824)	Sandstone	Leaf litter	CO	CR	WID
Inflectarius rugeli (Shuttleworth, 1852)	Both	Leaf litter	CO		WID
Inflectarius inflectus (Say.1821)	Sandstone	Leaf litter	CO		WID
Appalachina sayana (Pilsbry, 1906)	Both	Leaf litter	CO		NOR
Mesodon normalis (Pilsbry, 1900)	Sandstone	Leaf litter	LR	CR	GSM
Mesodon thyroidus (Say,1816)	Sandstone	Leaf litter	CO		WID
Mesodon zaletus (A. Binney, 1837)	Limestone	Leaf litter	CO	CR	WID
Neohelix albolabris (Say, 1816)	Both	Leaf litter	CO		WID
Patera appressa Say, 1821	Both	Rock structure	CO		WID
Stenotrema angellum Hubricht, 1958	Both	Leaf litter	CO		CKY
Stenotrema edvardsi (Bland, 1856)	Both	Leaf litter	CO		WID
Stenotrema hirsutum (Say, 1817)	Both	Leaf litter	CO		VWV
Stenotrema stenotrema (Pfieffer, 1819)	Sandstone	Leaf litter	CO		WID
Triodopsis anteridon Pilsbry, 1940	Both	Leaf litter	CO		VWV
Triodopsis tennesseensis (Walker & Pilsbry, 1902)	Both	Leaf litter	CO		WID
Xolotrema denotatum (Ferussac, 1821)	Limestone	Rotting wood	CO	CR	WID

species has its main affinities with the northern states as far north as Maine. In the southern Appalachians, it is a species of higher elevation hardwood forests, usually found under moist leaf litter (Hubricht 1985). At Bad Branch, the species was taken from three sites, found in the sandstone substrata among moist leaf litter. At one site, two shells of *S. ferrea* along with nine other species of snails were collected from moist leaf litter found in

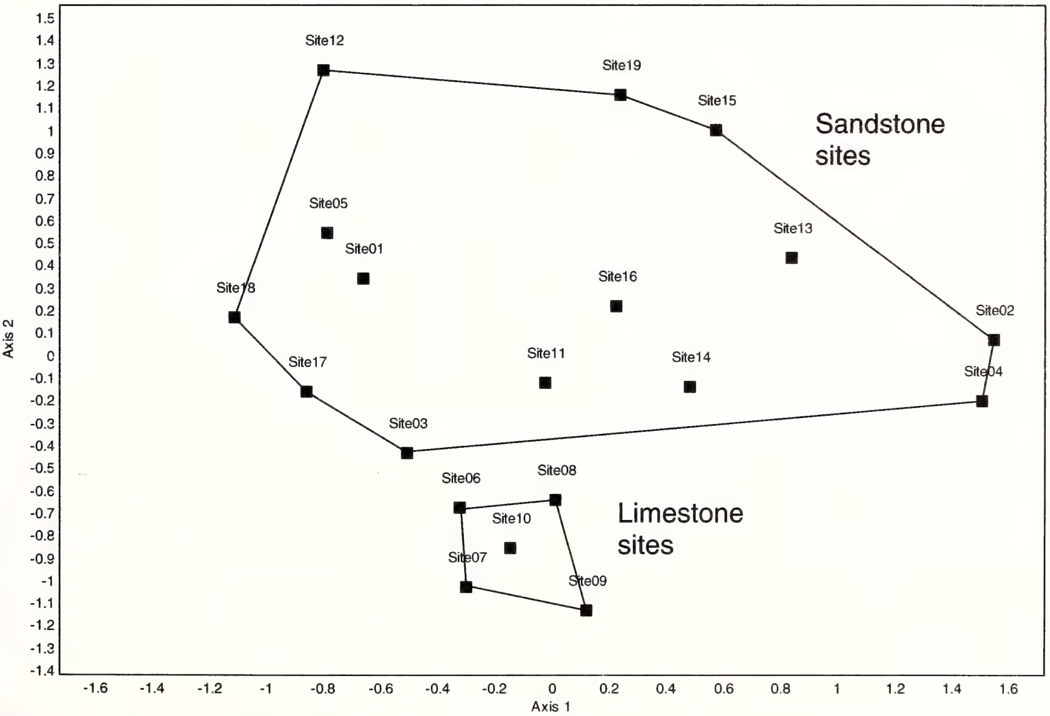

Figure 4. Non-metric multidimensional scaling ordination of nineteen sites where the land snail fauna was sampled at Bad Branch State Nature Preserve in Letcher County, Kentucky.

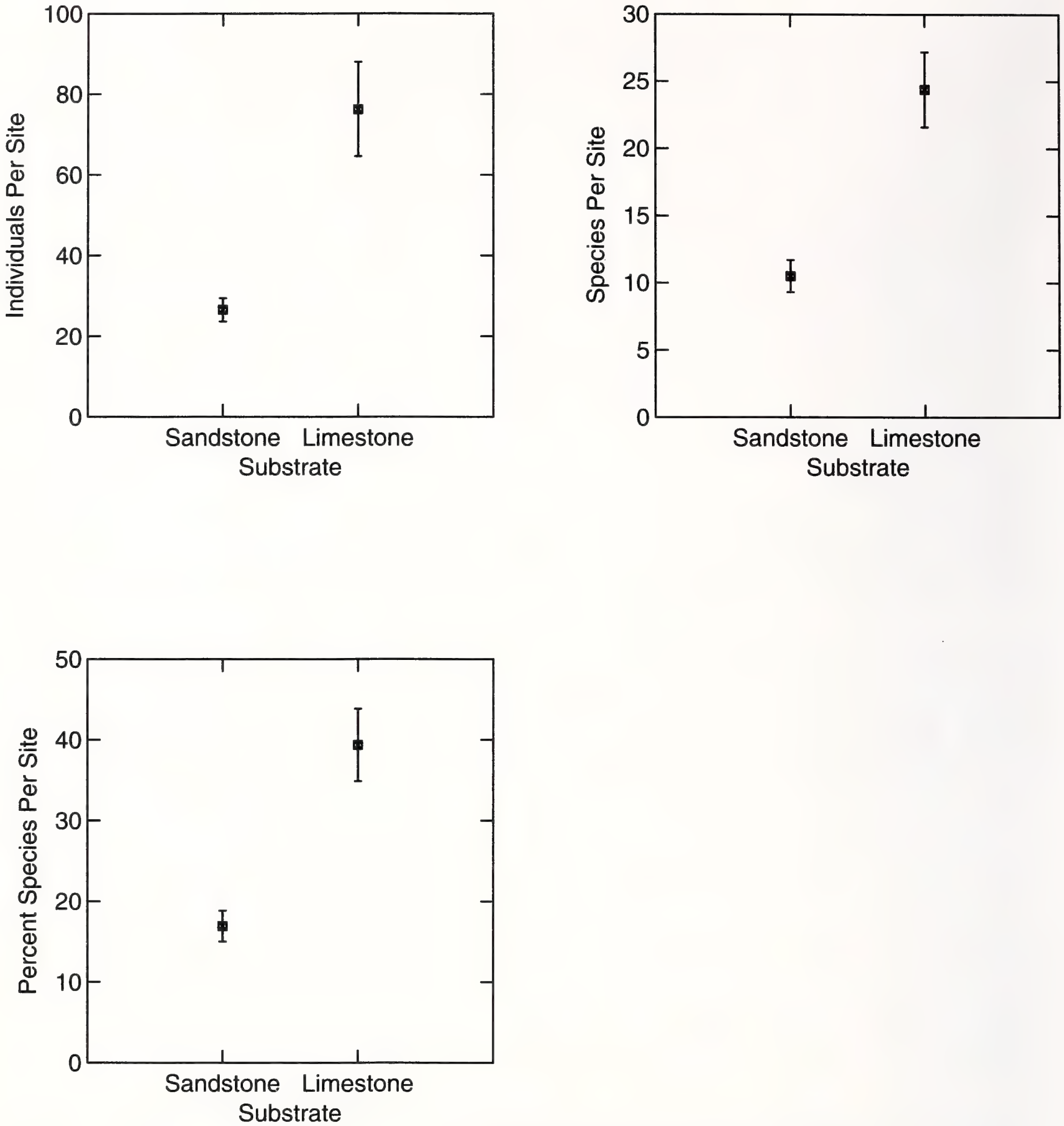

Figure 5. Abundance (individuals/site), species richness (species/site), and percent species/site of land snails on two substrates at Bad Branch State Nature Preserve in Letcher County, Kentucky.

the crotch of a forked poplar tree about a meter off the ground. Although this seems an uncharacteristic habitat for land snails, many single tree crotches in various eastern states have in fact harbored multiple species, as many as 15 taxa in rare cases. A study in the Great Smoky Mountain National Park showed that a number of infrequent land snails, (including an undescribed *Carychium* sp.) are occasionally found in tree crotches, sometimes positioned as high as 4 m off the ground (Dourson and Dourson 2006). Most of the snails found in these situations are micro species (<3 mm) including but not always limited to the genera *Carychium*, *Gastrocopta*, *Vertigo*, *Punctum*, *Striatura* and *Paravitrea*. Other snail species that are intermittently arboreal such as *Anguispira jessica* have been documented as high as 21 m in tulip trees found in the Great Smoky Mountains (Keller 2002).

A species occurring rather frequently in the Cumberland Plateau and on Pine Mountain in eastern Kentucky is *Appalachina sayana*. An

Figure 6. *Anguispira mordax*, unique for its prominent, widely spaced ribs and color features. It is one of twelve land snail species found at Bad Branch Nature Preserve that has biogeographical affinities with the Great Smoky Mountains. Two standard views are displayed; shells are 18 mm in diameter.

interesting anomaly found in the species has been inadequately discussed in past literature. Although considered a common snail in Kentucky, there are in fact two relatively distinct forms found in the state. Their shell morphologies are dissimilar enough to allow easy separation. One form of *A. sayana*, the larger and more common of the two is found on the Cumberland Plateau (here referred to as *A. sayana* CP for Cumberland Plateau, Figure 7a). The smaller form (here referred to as *A. sayana* PM for Pine Mountain, Figure 7b) is found on Pine Mountain. *A. sayana* CP averages 3–4 mm larger than *A. sayana* PM and usually displays both the basal and parietal tooth. *A. sayana* PM usually displays only the basal tooth and has a thin wire-like lip, remaining somewhat concave in shape its entire length. The umbilicus of *A. sayana* CP (Figure 8a) is umbilicate where as the umbilicus of *A. sayana* PM (Figure 8b) is more or less rimate. Although these two forms were considered the same species by Pilsbry (1940), Hubricht (1985), and others, *A. sayana* PM represents at the very least an interesting ecological form to the southeastern mountains of Kentucky.

Seven snail species that are of interest due to their infrequency or limited range in Kentucky include *Punctum vitrem* H. B. Baker, *Ventridens theloides: form, nodus* (Walker and Pilsbry), *V. collisella* (Pilsbry), *V. lawae*, *Philomycus venustus* (Figure 9), *Paravitrea subtilis* and *Hendersonia occulta*. Two species found near the Bad Branch parking lot, *Mesodon thyroidus* (Say) and *Inflectarius inflectus* (Say), although native to Kentucky, can be indicators that the natural vegetation has been disrupted, which is certainly the case for that site. Neither of these species was found beyond disturbance locales. The discovery of *Stenotrema angellum* at Bad Branch is an interesting anomaly given that the species primary range occurs in central Kentucky (Hubricht 1985). Specimens of *S. angellum* found at Bad Branch were smaller, more globose in profile, and generally more hirsute than *S. angellum* from central Kentucky.

DISCUSSION

In the early 1900s, Pilsbry considered the broad Valley and Ridge Provinces of eastern Tennessee to be a partial barrier to the intermingling of snail faunas occurring in the Cumberland Plateau (which at that time was more or less grouped with Cumberland and Pine Mountains) with snails from the area of Roan Mountain to the Great Smoky Mountains. Collections since by Branson and Batch (1967, 1968, 1988), Petranka (1982), Hubricht (1983), and others have ameliorated the barrier concept, revealing some interesting biogeographical associations. The principal land snail affinities of the southeastern mountains of Kentucky are in fact with the Great Smoky Mountain-Blue Ridge physiographic sections of the Cumberland Province, with some divisions of the snail fauna showing relationships with Virginia and West Virginia. Other examples of these broader biogeographical associations at Bad Branch include two small mammals, *Sorex dispar*, long-tailed shrew and *Microsorex thompsoni*, Thompson's pigmy shrew (Caldwell 1980). A small portion

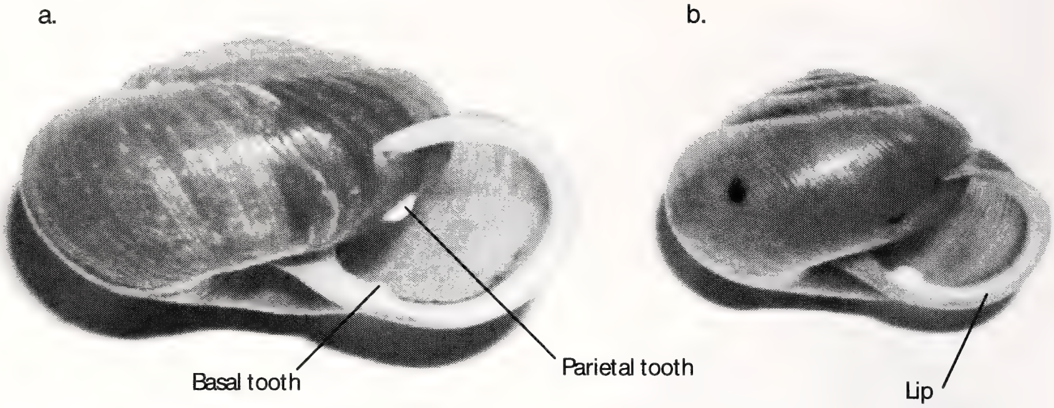

Figure 7a–b. Standard side view comparisons between two forms of *A. sayana* found in eastern Kentucky. The larger and more common of the two forms is found on the Cumberland Plateau (7a, shell diameter 26 mm), the smaller form (7b, shell diameter 16 mm) is found on Pine Mountain and at Bad Branch. Key features for separating the two forms include the parietal tooth (or the absence of it) and their relative size to each other.

of the Pine Mountain snails have their affinities with more northerly faunas. Of the 64 species found at Bad Branch, approximately eleven including *Paravitrea lamellidens* are representative of southern mountain ranges and eight including *Paravitrea dentilla* are representative with West Virginia and Virginia with around nine having their affinities with more northerly faunas (Table 1). Most of the remaining snails found at Bad Branch are considered to be relatively wide-ranging species across eastern North America, showing no close affiliation for a specific region.

Besides the interesting biogeographical associations found at Bad Branch, the preserve also harbors a rich snail fauna, a result of several geophysical and ecological factors. Land snail abundance, both in terms of diversity of species and numbers of shells, is often highly correlated with increasing soil or litter pH (Burch 1955; Valovirta 1968; Wareborn 1970; Hotopp 2002), soil moisture

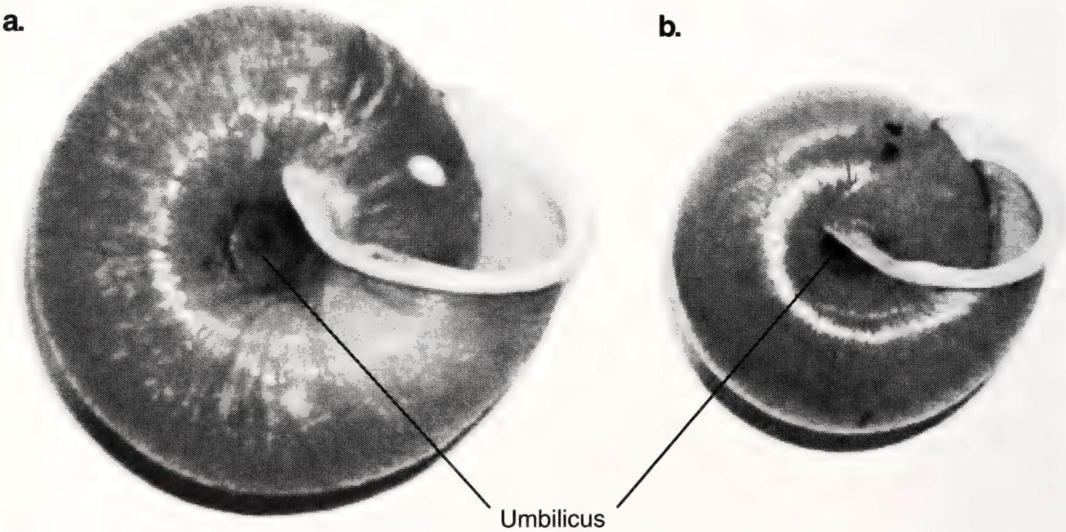

Figure 8a–b. Standard bottom view comparisons between the two forms of *A. sayana* found in eastern Kentucky, the larger and more common of the two is found on the Cumberland Plateau (8a , shell diameter 26 mm), the smaller form (8b, shell diameter 16 mm) is found on Pine Mountain and at Bad Branch. Bottom views show several key features for separating shells including the umbilicus, lip and their relative size to each other.

Figure 9. *Philomycus venustus* is one of several land snail species found at Bad Branch Nature Preserve that have biogeographical affinities with the Blue Ridge, specifically the states of Virginia and West Virginia. Length of animal while crawling is 70 mm.

(Boycott 1934; Getz 1974; Pollard 1975) and soil cations, particularly calcium (Burch 1956; Agocsy 1968; Atkins 1968; Petranka 1982). Calcium carbonate is required by land snails for regulation of bodily processes, reproduction, but most importantly shell-building (Burch 1962; Fournie and Chetail 1984; Hickman et al. 2003). Litter Ca and salt types can directly increase growth rates and fecundity in some species (Wareborn 1969, 1970, 1979). As would be expected, land snail scarcity is associated with low soil pH Burch (1955), declining soil cations, specifically Ca (Petranka 1982), increasing coniferous presence (Jacot 1935; Karlin 1961), and increasing elevation (Petranka 1982). The influence of pH on land snails is thought to be indirect, its main effect being a lowering of the amounts of soil cations, principally Ca (Karlin 1961; Cameron 1970).

Calcium occurs in abundance on the north slope of Pine Mountain in the form of limestone outcrops, screes, and finally, the soils. The highest number of shells (454) were found here and are largely correlated to the calcium rich soils and to a lesser degree slope gradient, but in terms of species diversity, the limestone sites (49 species) were nearly equal to the sandstone sites (43 species). These results were not surprising given the large difference in samples between sandstone and limestone (almost three to one). As a result, we felt that conventional diversity indices were not appropriate for the analyses. Nevertheless, the analyses we did use clearly showed greater diversity and different species composition between the two substrates when standardized by site. There is a relationship between shell abundance and species richness, so in one sense, it is not surprising that richness was so much greater on limestone than sandstone (because there were so many more individuals on limestone). But this is much more than a sampling artifact, as there is also a relationship between area and species richness. Given that sandstone had nearly 3 times the number of samples as limestone (hence almost 3 times the area sampled), it would have been expected a greater total number of individuals and species on sandstone if it had more or less the same levels of diversity as limestone. This was not the case, and it speaks even more to how much greater diversity is on limestone than sandstone. From a management standpoint, protected areas on sandstone substrata would need to be larger in order to preserve similar snail diversity found in limestone. Because many of the calciphile species found on limestone will not likely occur on sandstone and vice-versa, protecting substrata diversity, at least in terms of sustaining snail multiplicity is equally important.

Another factor that has likely increased, to some extent, the numbers of snails on the steep north slopes of Pine Mountain was the slope gradient. Coney et al. (1982) found more species of land snails on steep slopes than on more moderate ones. Petranka (1982) found that 15 of the 56 land snail species found on Black Mountain showed some preference for slope, with 9 species showing an affinity for increasing slope. The importance of leaf litter moisture (thought to be a factor of slope) to land snails was emphasized by Boycott (1934), Getz (1974), Pollard (1975), and others. Although aspect was reported to markedly affect microclimate (Braun 1940, 1942; Geiger 1965), Petranka's (1982) study found no environmental variable to be significantly correlated with aspect. With respect to elevation, Petranka (1982) reported that pH, potassium, calcium, and magnesium levels would decrease (ppm) with increasing elevation and that the number of snail species and the number of individuals found per site would also decrease with elevation. The results of our work showed no particular trend as a function of elevation. Petranka's (1982) study plots (from 335 m to 1261 m), however, had more than double the elevation

ranges from which to sample than was found at Bad Branch (from 457 m to 914 m).

Clearly, there have been a number of studies on snail abundance or their scarcity as it relates to soil cations, gradient, elevation, vegetation, and litter moisture. Less well-known is the effect that large geophysical landscape edges have on driving biodiversity, particularly in land snails. The Central Knobstone Escarpment in Powell County, Kentucky, forms a large geophysical landscape edge on Furnace Mountain where the Cumberland Plateau, the Knobs, and the Outer Bluegrass regions of Kentucky converge. The merging of these distinct regions were shown to have an exceptional number of land snails, a reported 61 species found co-existing within a 2-ha mesic hillside (Dourson 2007). Analogous to Furnace Mountain, Bad Branch State Nature Preserve also forms a great "geophysical landscape edge" located along the Pine Mountain massif of southeastern Kentucky, bordered to the south by the Valley and Ridge Provinces and to the north by the Cumberland Plateau. All three regions contain their own snail affiliations. Moreover, the amalgamation of these snail rich eco-regions has provided a number of terrestrial gastropods an opportunity to coexist. This large landscape edge, together with the wide range of soil types, elevation and slope gradient has brought together an interesting and rare assemblage of species not frequently occurring in the state of Kentucky. While the majority of land snails found at Furnace Mountain study were by and large common and wide ranging species, the snails at Bad Branch showed tighter affiliations with a particular region of eastern North America and were generally rarer land snail species.

When compared with other collections made in southeastern Kentucky, Bad Branch exceeded their reported numbers. The most limited collection was by Pilsbry (1940) who secured 22 land snail species (unknown numbers) from a single locality in Harlan County, Kentucky. Branson and Batch (1968) reported 47 species (528 individuals) in Bell (3 sites) and Harlan (1 site) counties. A number of their snail accounts however, may have been erroneous. In Kentucky for example, *Triodopsis fosteri* (F. C. Baker) and *Webbhelix multilineata* (Say), two species reported in their collections, are restricted to counties along the Ohio River (Hubricht 1985). Other questionable species that Branson and Batch (1968) reported from Black Mountain included *Glyphyalinia virginica* (Morrison) (an endemic to northern Virginia) and *Triodopsis fraudulenta* (Pilsbry) (reported from eastern West Virginia, northern Virginia, and a few counties in Pennsylvania). Branson and Batch (1968) also reported one exotic slug *Deroceras reticulatum*, a native to Europe. The actual number of indigenous snails from their study is probably closer to 40 species. Petranka (1982) reported 56 land snails (12,464 individuals) at 36 sites from Big Black Mountain in Harlan County, Kentucky and Wise County, Virginia, adding 21 new county records for Harlan. Petranka's study covered the largest and most extensively sampled area to date in the southeastern mountains of Kentucky. Hubricht et al. (1983) reported 43 species from scattered locations across Black and Pine Mountains in Harlan County. Snail collections made by Branson and Batch (1968), Petranka (1982) and Hubricht et al. (1983) covered larger sample areas than our study.

Further investigations at Bad Branch State Nature Preserve no doubt will add a few more taxa. Additions would most likely include *Vertigo bollesiana* (Morse), *Mesomphix rugeli* (W. G. Binney), *Zonitoides elliotti* (Redfield), *Vitrinizonites latissimus* (Lewis), *Neohelix dentifera* (A. Binney), *Triodopsis tridentata* (Say), *Triodopsis vulgata* Pilsbry, *Triodopsis juxtidens* (Pilsbry), *Strobilops aenea* Pilsbry, *Philomycus togatus* (Gould), *Pallifera secreta* (Cockerell) and *Discus nigrimontanus* (Pilsbry). These species are reported from Harlan County on Pine and Black Mountains (Pilsbry 1940, 1946, 1948; Hubricht 1983, 1985). A number of these land snails also have their affinities with the Great Smoky Mountain-Blue Ridge sections of the Cumberland Province, and Bad Branch has certainly demonstrated these same affinities. The real number of land snail species at Bad Branch State Nature Preserve is probably closer to 70.

ACKNOWLEDGEMENTS

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Molecular and Morphological Evidence for the Occurrence of Two New Species of Invasive Slugs in Kentucky, *Arion intermedius* Normand and *Arion hortensis* Férussac (Arionidae: Stylommatophora)

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ABSTRACT

Arion intermedius Normand and *Arion hortensis* Férussac are invasive mollusks in North America, having been previously reported in thirteen and seven U.S. States, respectively. We report here the first records of both species in Kentucky. Because slug species within the genus *Arion* Férussac show high degrees of intra-specific variation, identifications were confirmed using both morphological and molecular (partial COI gene sequences) methods. These new records are of concern because invasive slugs are major pests in agriculture, horticulture and floriculture, causing considerable damage to wheat, alfalfa, corn, soybean and tobacco that are amongst the most economically important crops in Kentucky. This study also highlights the important need for additional gastropod surveys throughout the U.S.A.

KEY WORDS: slugs, *Arion hortensis*, *Arion intermedius*, invasive species, agroecology, molecular diagnosis

INTRODUCTION

With the recent increases in global trade, the introduction of exotic gastropods continues to pose serious problems throughout North America (Robinson and Slapcinsky 2005). Robinson (1999) listed 4,900 gastropod interceptions at U.S. ports from nearly 100 countries between 1993 and 1998. These records comprised 369 species from 197 genera in 71 families. Although the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS) screens shipments at U.S. seaports, airports, and border crossings, it is inevitable that some gastropods make it through due to limited human resources (Robinson 1999) and to the cryptic nature of many species, especially the small size of their eggs and hatchlings. There currently are over 80 non-native snail and slug species with established, self-sustaining populations in the U.S. and Canada (excluding the Hawaiian Islands and Puerto Rico). Many of these species pose a significant threat to horticultural and agricultural industries in addition to the natural environment (Robinson and Slapcinsky 2005).

However, according to Reise et al. (2006), little has been published on introduced terrestrial gastropods within the U.S. over the past two decades. This is of concern because such introductions tend to dominate the malacofauna in anthropogenic habitats and as a result tend to be economically important as pests of agriculture and horticulture. Robinson (1999) suggested that surveys of urban and suburban areas by malacologists will be crucial in identifying additional invasive gastropods and should enable mitigation measures to be implemented more rapidly, thereby preventing the establishment

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Figure 1. *Arion intermedius* Normand from Berea College Forest, Madison County, Kentucky.

of pestiferous species and consequently reducing eradication costs.

In response to the need for faunistic evaluations of invasive gastropods, a survey was conducted in urban, suburban, agricultural, horticultural, floricultural and natural areas throughout Kentucky to document the native and invasive slug species and to ascertain the potential impact upon agricultural production. The surveys were designed to complement the existing knowledge of the slug fauna of Kentucky (Burch 1962; Branson and Batch 1969; Dourson and Feeman 2006) and resulted in the discovery of the first specimens of *A. intermedius* (Figure 1) and *A. hortensis* (Figure 2) in the state.

METHODS AND MATERIALS

Slug collections were made throughout Kentucky by hand collecting and using simple baited traps (Friskies® canned cat food covered with a black plastic refuse bag pinned at each corner and left overnight). Specimens were kept alive in small plastic containers lined with damp paper towels and fed on organic carrots. On return to the laboratory, specimens were identified using diagnostic morphological characteristics (outlined below) and then preserved in 100% ethanol. Because slugs, especially species within the genus *Arion* Férussac, show high degrees of intraspecific variation (Pinceel et al. 2004), species-level identification was confirmed using molecular methods.

A portion of foot tissue was excised from 14 specimens of *A. intermedius* and the one individual of *A. hortensis*. DNA was then extracted using a Qiagen® commercial extraction kit (Tissue protocol) (Qiagen Inc., Valencia, CA). Cell lysis was achieved overnight in a 56°C constant temperature shaker with proteinase K and Qiagen lysis buffer. The QIAamp Tissue Kit then adsorbed the DNA onto a silica membrane in a spin column that was cleaned with two wash buffers. Finally, purified template DNA was eluted in AE storage buffer. Using this DNA, a polymerase chain reactions (PCR) was used to amplify a 655 bp fragment of the mitochondrial cytochrome oxidase subunit I gene (Table 1). Amplified DNA was cleaned using the Wizard ® PCR Preps DNA Purification System (Promega, Madison, WI). The PCR product was first purified using a purification buffer and resin, and then washed using 80% isopropanol. Clean DNA was eluted in double distilled water and then direct sequenced in both directions at the University of California, Riverside Genomics Institute Core Instrumentation Facility using an Applied BioSystems

Figure 2. *Arion hortensis* Férussac from an urban garden in Lexington, Fayette County, Kentucky.

3730 DNA Analyser with a Big-Dye ® V3.1 kit (Applied Biosystems, Foster City, CA). Raw sequences were trimmed by removing the primers and aligned manually in Bioedit v 7.0.5.3 (Copyright© Hall 1997–2004). Using these aligned sequences, a BLAST search on GenBank was performed to confirm our initial, morphological identifications (Table 2). Sequences are deposited in GenBank under accession numbers EU382742 for *A. hortensis* and EU382743–EU382756 for *A. intermedius*.

COLLECTION DETAILS

A total of 97 individuals of *A. intermedius* (Figure 1) were collected at two locations in the central Bluegrass region of Kentucky. The first location was Berea Forest (37°60′N, 84°25′W) in Madison County on 13 and 16 Apr 2007 (n = 70) and the second was

Table 1. Primer detail, reaction ingredients, and cycling conditions used for PCR amplification of the mitochondrial cytochrome oxidase subunit I gene fragment (655 bp) for *Arion intermedius* Normand and *Arion hortensis* Férussac. All reactions were carried out in 25 µl volumes (including 2 µl of template DNA).

Primer	Primer Sequence 5′-3′	PCR Reaction Ingredients	PCR Cycling Conditions
LCO1490 (Folmer et al. 1994)	GGTCAACAAATCATAAAGATATTGG	1 × PCR buffer (contains 2 mM $MgSO_4$, NEB, Ipswich, MA, U.S.A.) 20 µM of each dNTP	(i) 5 min at 95°C (ii) 40 cycles of 1 min at 95°C, 1 min at 40°C and 2 min at 72°C (iii) 5 min at 72°C
HCO2198 (Folmer et al. 1994)	TAAACTTCAGGGTGACCAAAAAATCA	0.2 µm of each primer 1 unit Taq (NEB) 1 mM $MgCl_2$ 1.25 µl BSA detergent	

Table 2. Molecular confirmation (BLAST) of the morphology based identifications for *Arion intermedius* Normand and *Arion hortensis* Férussac using a 655 bp fragment of the cytochrome oxidase subunit I mitochondrial gene.

			BLAST Results		
Species	Location	Specimens	E-value[2]	Identification[3]	Ascension Numbers
Arion hortensis	Urban Garden	1	0.0	100%	EU382742
Arion intermedius	Berea Forest	1–10[1]	0.0	100%	EU382743–EU382752
	Stonewall Park	1–4[1]	0.0	100%	EU382753–EU382756

[1] There were no nucleotide polymorphisms within the COI sequences for these individuals.
[2] The E-value is a measure of the random background noise that exists for matches between sequences. The lower the E-value the more "significant" the match between the test sequence and the matching Genbank sequence.
[3] The higher this percentage the greater the likelihood that the test species is the same as that predicted by the BLAST search.

Stonewall Park (n = 4) in Lexington, Fayette County. Berea Forest is a secondary oak-maple forest interspersed with small stands of hickory, pine and mountain laurel. Specimens were predominantly collected under discarded carpet close to an abandoned shack (n = 66). A smaller number (n = 4) were found under dead wood; further surveys on 8 May 2007 captured additional specimens (n = 23) under wood of fallen trees on the edge of the forest floor and adjacent to other collection locations. Other species collected with *A. intermedius* were *Deroceras laeve* (Müller) and *Deroceras reticulatum* (Müller).

Stonewall Park (38°00′117″N, 84°33′248″W) is a suburban park with managed and unmanaged grassland interspersed with stands of mixed deciduous trees. A stream also runs through the area. Specimens of *A. intermedius* were collected on 16 Apr 2007 under plywood and decaying logs. *Deroceras laeve* and *D. reticulatum* also were collected with *A. intermedius* in Stonewall Park.

A single specimen of *A. hortensis* (Figure 2) was collected under broken wood on a lawn in an urban garden in Lexington, Fayette County, Kentucky, on 26 Mar 2007 (37°59′3311″N, 84°29′2597″W). The only other species collected with this individual was *D. reticulatum*.

MORPHOLOGICAL IDENTIFICATION

Arion intermedius is a small slug up to 25 mm long (Barker 1999). The specimens that we collected in Kentucky were all grayish-yellow with distinctly darker tentacles (Figure 1) and yellow body mucus. The species is best separated from other slugs by the presence of small spikes on the body tubercles that give it a prickly appearance when contracted (Kerney and Cameron 1979). Internally, the genital atrium lacks a stimulator (Quick 1960).

Arion hortensis is a small slug up to 50 mm long. The body color tends to be dark blue-black (Figure 2), but the sole and body mucus are yellow to orange and sticky (Barker 1999). According to Pfleger (1999) the tip of the tail is the same color as the mucus. However, Davies (1977, 1979) confirmed that *A. hortensis s.l.* is a species complex comprising *A. hortensis s. s.*, *Arion distinctus* Mabile, and *Arion owenii* Davies. The three species are difficult to distinguish on external characters alone, and for that reason dissection and/or molecular analysis should be used to confirm identifications. Because *A. distinctus* has been reported in other U.S. states (Mc Donnell et al. 2009), it is possible that it also occurs in Kentucky. We therefore provide details of how to reliably identify both species. According to Backeljau and Van Beeck (1986), the shape of the epiphallus structure (i.e., the structure associated with the outlet of the epiphallus in the genital atrium) is the most reliable diagnostic character. In *A. distinctus*, it is conical, protrudes into the atrium, and covers the outlet of the epiphallus. A gutter or fissure runs from the margin of the epiphallus structure to its centre (Backeljau and Van Beeck 1986). In *A. hortensis* this structure is a relatively inconspicuous, oblong plate that covers about half of the epiphallus outlet. It never has a gutter/fissure (Backeljau and Van Beeck 1986). *Arion owenii* appears to be the rarest member of the complex (Backeljau and Van Beeck 1986), and there are no records from the U.S (Mc Donnell et al. 2009). The epiphallus structure in this species is variable but it is generally, long, slender, tongue-like and it protrudes from the outlet of the epiphallus.

DISTRIBUTION AND ECOLOGY

Although the native range of *A. intermedius* is central and western Europe (Barker 1999), it is found as an exotic throughout the world: the Azores (Quick 1960), Australia, Europe, New Zealand, North America, South Africa (Barker 1999), Canada, North Africa, and Polynesia (Forsyth 2004). In the U.S. it has been collected in California, Connecticut, Hawaii, Idaho, Maine, Massachusetts, Michigan, New Hampshire, New York, North Carolina, Rhode Island, Tennessee, and Washington (Chichester and Getz 1969; Dundee 1974; Pearce and Blanchard 1992; Pearce and Bayne 2003; Mc Donnell et al. 2009). *Arion intermedius* is known to frequent hay fields (Bruijns et al. 1959), grasslands (Cameron 1978; Lutman 1978), gardens (Forsyth 2004), pasture (Quick 1960) and, to a lesser extent, disturbed agricultural cropping systems (e.g., Glen et al. 1984). In central and western Europe, where it is native (Barker 1999), it also inhabits forests (Bishop 1977; Tattersfield 1990) and feeds on fungi. Chichester and Getz (1969) reported this species from mixed and deciduous forests in the Great Smoky Mountains of Tennessee and North Carolina. According to Barker (1999) and Glen et al. (1984), the species may not be pestiferous, but Christian et al. (1999) cited it as a pest of wheat, and Barker (2002) highlighted it as a pest of pasture due to its effects on plant productivity.

Arion hortensis is thought to be native to western and southern Europe (Roth and Sadeghian 2006), but it also is present as an exotic in other parts of the world. However, because it belongs to a species complex, pre-1977 records of this slug need to be treated with caution as authors did not distinguish between *A. hortensis* s.s., *A. distinctus*, and *A. owenii*. Nevertheless, it has been collected throughout Europe, North America, and New Zealand in recent times (Barker 1999). In the U.S. it is known from California, Hawaii, Idaho, Maine, Michigan, Pennsylvania and Washington (Pearce and Blanchard 1992; Pearce and Bayne 2003; Mc Donnell et al. 2009). This species commonly is associated with disturbed habitats including urban areas such as gardens, roadsides, and forest fringes (Barker 2002). It is a known pest of a wide range of vegetable crops (reviewed by South 1992; Barker 1999), horticultural crops (South 1992), pasture (Barker 2002), and sunflowers (Ballanger and Champolivier 1996).

DISCUSSION AND CONCLUSIONS

Given the dearth of publications on the invasive slug fauna of North America in the last 20 years, it is imperative that discoveries of new species are published (Mc Donnell et al. 2008). First, such reports are important as they provide a baseline for future studies involved in monitoring the subsequent survival and spread of pest populations. Second, the current lack of information on the distribution of such invasive species means that USDA-APHIS lacks the necessary information to determine if an intercepted slug species represents a potential new threat to agriculture, horticulture, and the natural environment (Reise et al. 2006).

Our new records are of concern primarily because invasive slugs are major pests in agriculture, horticulture, and floriculture (South 1992), causing considerable damage to important crops such as wheat (Martin and Kelly 1986; Kemp and Newell 1987), alfalfa (Grant et al. 1982; Barratt et al. 1989), corn (Mallet 1973; Barratt et al. 1989), soybean (Hammond 1985; Barratt et al. 1989), and tobacco (Mistic and Morrison 1979). These crops are the five most economically important for Kentucky, and the confirmation of two new invasive slug species in the state is of obvious concern to the $3.97 billion farm commodity industry (USDA – NASS Kentucky Field Office 2006). Furthermore, organic growers rank slugs and birds as the second most important pests after weeds (Peacock and Norton 1990). With the drive towards agricultural diversification and low-input production, the planting of high value and highly vulnerable commodities such as strawberries could lead to yield loss due to known slug feeding habits within such crops (Prystupa et al. 1987; Duval and Banville 1989). While the most effective means of control is often molluscicides (Hammond et al. 1996), alternative approaches such as cultural and biological control need further examination to accurately evaluate their role in management programs in a variety of systems.

Finally, it is imperative that faunistic surveys are undertaken in other states and provinces throughout North America, particularly those where the slug fauna is essentially undocumented e.g., North and South Dakota. This need also is reflected in the disjunct distribution of both *A. intermedius* and *A. hortensis* in the U.S. It is unlikely that both species are confined to the states mentioned above and more likely that a continuous distribution exists, which is likely to be confirmed with further malacological surveys. It is only by carrying out such surveys that the distribution of exotic gastropods within the U.S. will be clarified and the paucity in knowledge of these invasive pests improved. Such knowledge will be essential in mitigating the potentially severe damage to agricultural and horticultural systems caused by these pests, in addition to helping curb their spread to other states from potential hotspots. We also suggest a more widespread use of molecular methods for confirming the identity of invasive gastropods, particularly those in enigmatic genera such as *Arion*. The provision of such information may help to uncover additional species complexes in addition to providing potentially useful information for the elucidation of invasive pathways, both of which will be essential for successful control.

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Inventory and Analysis of the Pteridophytes of Carter Caves State Resort Park, Carter County, Kentucky

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ABSTRACT

Carter Caves State Resort Park (CCSRP) is comprised of 800 ha in Carter County, Kentucky. A field-based inventory of the pteridophytes of the park was conducted from September 2005–October 2007. Additionally, pteridophyte collections at regional herbaria were examined for specimens from the park. These efforts yielded a pteridophyte flora of 46 species and one hybrid for CCSRP; this is 61% of Kentucky's known pteridophyte flora. Index of similarity values with other published floras ranged from 22.2% to 84.5%. CCSRP had over twice the predicted number (21) of pteridophyte species based on a species-area curve constructed with data from other regional floras.

KEY WORDS: Carter Caves, Cliff Section, ferns, pteridophytes, taxonomy

INTRODUCTION

Carter Caves State Resort Park (CCSRP) lies within the Cliff Section in the northern portion of the Cumberland Plateau region of Kentucky and is located about 8.0 km north of Olive Hill in western Carter County (Figure 1). The park centroid is located at latitude 38.3713°N and longitude 83.1175°W.

The park is regionally and nationally known for its cave systems. Numerous state rare vascular plant species are known from CCSRP including *Acer spicatum* Lam., *Paxistima canbyi* A.Gray, *Thaspium pinnatifidum* (Buckley) A.Gray, *Taxus canadensis* Marsh., and *Viola walteri* House. Other botanical inventory work in the Cumberland Plateau has typically included entire vascular floras (e.g., Wofford et al. 1979; Sole et al. 1983; Thompson and Fleming 2004). Huffaker (1975) conducted a vascular plant inventory along Tygarts Creek upstream, adjacent, and downstream of CCSRP and reported 25 pteridophyte species. A 1939 Spring Foray of the Southern Appalachian Botanical Society that included many notable botanists (e.g., E. Lucy Braun and Earl Core) took place in CCSRP, but only two pteridophyte species were cited from the park (Gilbert 1939). Despite its reputation for botanical diversity, no botanical inventories are known to have been conducted in CCSRP.

Publications pertaining solely to pteridophytes in Kentucky are Williamson (1878), McCoy (1938), and Cranfill (1980). Williamson (1878) listed 40 species of pteridophytes from Kentucky with two species from Carter County. McCoy (1938) presented county distributions for 62 species, lesser taxa, and hybrids in Kentucky, including 32 from Carter County. Cranfill (1980) listed 70 pteridophyte taxa for Kentucky with 46 species and hybrids as occurring in Carter County. The same number of pteridophyte taxa was listed by Campbell and Medley (2006) for Carter County. The purpose of this study was to inventory Carter Caves State Resort Park (CCSRP) for pteridophytes and compare these results with those of other published floras.

STUDY AREA

CCSRP is located on the Wesleyville (Philley and Chaplin 1976), Tygarts Valley (Sheppard 1964), Grahn (Englund 1976), and Olive Hill (Englund and Windolph, Jr. 1975) 7.5′ quadrangles. The park is divided into two separate tracts of land with the major segment of the park being to the northwest of the smaller section (Figure 2).

The larger section contains most of the buildings found within the park, including a lodge, welcome center, cabins, park residences, and maintenance buildings. This section also includes a golf course and an 18.3 ha lake. Horn Hollow projects from the park to the northeast and is relatively isolated from the

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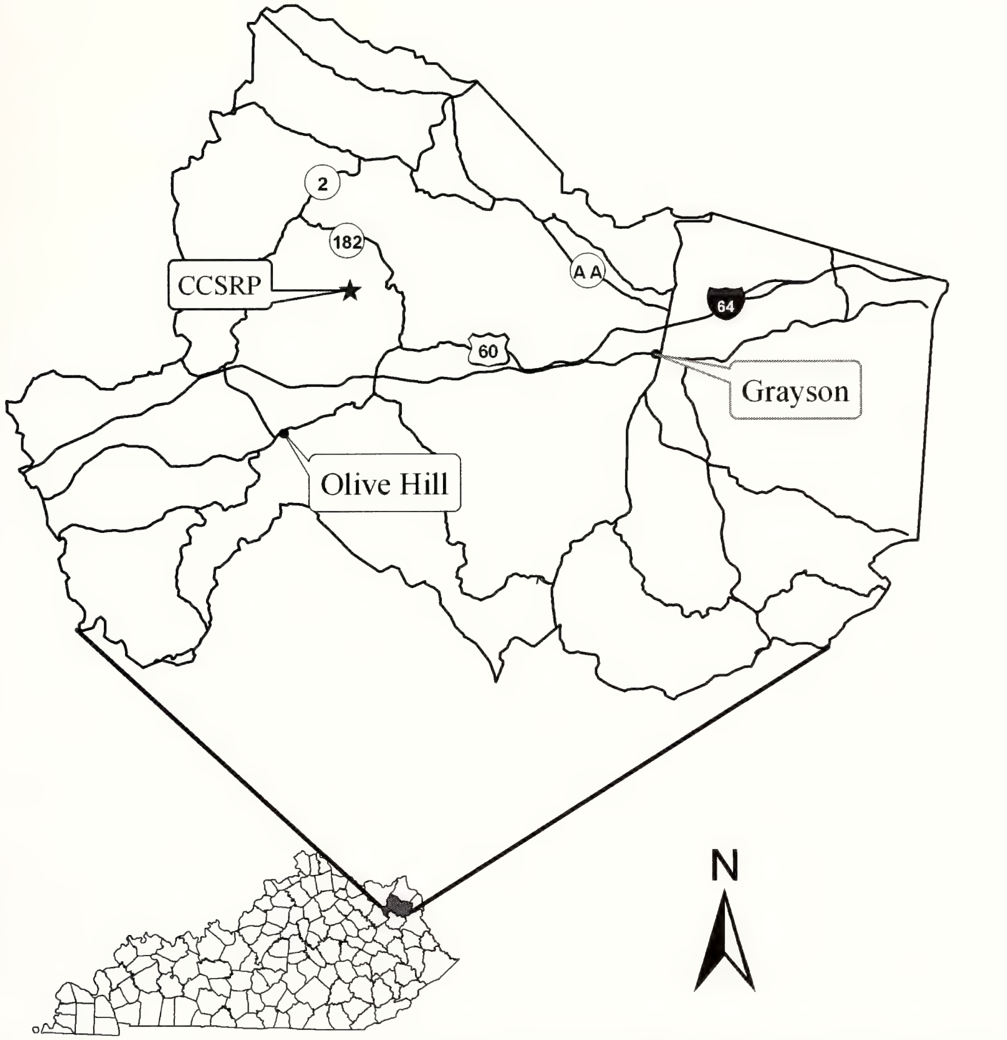

Figure 1. Map showing location of Carter Caves State Resort Park (CCSRP) within Carter County, Kentucky.

major infrastructure of the park. Two state nature preserves lie within CCSRP: Bat Cave State Nature Preserve comprises the northwestern corner of the park and Cascade Caverns State Nature Preserve is located in the Cascade Caves portion of the park.

Elevation overall ranges from 219.5–341.4 m (720–1120 ft), with the maximum relief in any one area being 79.2–91.4 m (260–300 ft). The park contains a very weather resistant sandstone layer at its highest elevations, and below this lies a very weatherable limestone layer. The sandstone layer is comprised of two formations. The upper layer is the Breathitt Formation, which supports dry upland communities. The lower layer is the Lee Formation, which often outcrops as cliffs (Sheppard 1964; Englund and Windolph, Jr. 1975; Englund 1976; Philley and Chaplin 1976). Cliffs and their overhangs support a number of pteridophyte species, many of which are restricted to these habitats. The dominant woody species of these dry uplands are *Quercus coccinea* Münchh., *Q. alba* L., *Acer rubrum* L., *Carya* spp., *Nyssa sylvatica* Marshall, *Vaccinium pallidum* Aiton, and *Smilax rotundifolia* L. Dominant species immediately below the bases of these cliffs are *Betula lenta* L., *Tsuga canadensis* (L.) Carr., and *Rhododendron maximum* L.

Figure 2. Topographic map of Carter Caves State Resort Park. The park is located on four 7.5′ topographic quadrangles: I-Wesleyville, II-Tygarts Valley, III-Olive Hill, IV-Grahn.

The Pennington and St. Genevieve members of the Newman Limestone lie beneath the Lee Sandstone (Sheppard 1964; Englund and Windolph, Jr. 1975; Englund 1976; Philley and Chaplin 1976). Dominant species in calcareous uplands are *Quercus muhlenbergii* Engelm., *Acer saccharum* Marshall, and *Fraxinus americana* L. Species dominating the mid to lower slope communities are *Acer saccharum*, *Quercus alba*, *Liriodendron tulipifera* L., *Fagus grandifolia* Ehrh., and *Acer rubrum*.

The lowermost geologic material exposed within the park beneath the St. Genevieve Limestone is the Cowbell Member of the Borden Formation. This rock is composed of

shales (Sheppard 1964; Englund and Windolph, Jr. 1975; Englund 1976; Philley and Chaplin 1976). The Borden Formation has little exposure within the park but forms the bedrock of the lower reaches of Cave Branch and Smoky Creek and constitutes the bedrock of Tygarts Creek within CCSRP.

MATERIALS AND METHODS

Thirty nine collecting trips were made to CCSRP from September 2005 through October 2007. A concentrated effort was made to thoroughly examine all representative habitats within the park. Sixty-five pteridophyte collections were made. All specimens collected were deposited in the Morehead State University Herbarium (MDKY). Additionally, the entire pteridophyte collections in MDKY and the herbaria of Eastern Kentucky University (EKY), University of Cincinnati (CINC), Marshall University (MUHW), and the University of Kentucky (KY) were examined for specimens from CCSRP. A literature search for pteridophyte collections within CCSRP was conducted which led to the examination of a Carter County, Kentucky, specimen of *Adiantum capillus-veneris* L. from the Gray Herbarium (GH).

Specimens were tentatively identified in the field and then further examined using microscopes in the laboratory for definitive determinations. Cranfill (1980), Flora of North America Editorial Committee (1993), and Jones (2005) were used for identification. Taxonomy and nomenclature follow the Flora of North America Editorial Committee (1993).

The relative abundance of each species in CCSRP (Table 1) was estimated based on field observations and categorized as Common - characteristic and dominant in many habitats; Frequent - generally encountered; Infrequent - scattered localities, populations usually small; and Rare - one to three populations known.

A species area curve was constructed using other published floras from Kentucky and nearby regions for pteridophytes (Wade and Thompson 1991). SPSS 15.0 was used to $\log_{10}$ transform pteridophyte species numbers and the study area sizes in hectares, and a power function model was used to create a line of best fit (Figure 3).

Sørenson coefficients of similarity, based only on pteridophytes, were calculated with other published or available floras that included pteridophytes in the Appalachian Plateau as well as with selected floras from other regions of Kentucky, Tennessee, Ohio, North Carolina, Virginia, and West Virginia. Sørenson's coefficient was calculated by using the formula outlined in Sneath and Sokal (1963) as 2W/ (A+B), where W is the number of species the two floras have in common, A is the total number of species in the first flora, and B is the total number of species from the second flora. Only species and hybrids were used in these comparisons; infraspecific taxa were excluded from the calculations.

RESULTS

Forty-six pteridophyte species and one hybrid were documented for CCSRP. Forty-four of these were found in the field, two (*Asplenium Xwherryi* D. M. Smith et al., *Botrychium biternatum* (Savigny) Underw.) were in MDKY, and one (*Adiantum capillus-veneris*) was in GH. The largest families were Dryopteridaceae with twelve representatives and Aspleniaceae with seven representatives. The largest genera found were *Asplenium* with seven representatives and *Cystopteris*, *Dryopteris*, and *Osmunda*, each with three members (Table 1).

Examination of collections in CINC, EKY, KY, MDKY, and MUHW yielded 74 additional CCSRP specimens. Of the herbaria visited, only three (MDKY, KY, MUHW) contained collections from CCSRP. MDKY had 52 specimens representing 26 different species and one hybrid. KY housed 15 specimens totaling 13 species. In MUHW there were seven specimens accounting for four species from CCSRP. None of the species at the latter two herbaria represented taxa not documented by field work during the present study. McCoy (1938) listed an *Adiantum capillus-veneris* specimen collected by a G. G. Jr. (full name unknown) in 1899 housed in GH from the Carter Caves region of Carter County. Examination of this specimen verified the identification.

Some pteridophytes showed an exclusive relationship with either sandstone or limestone. Those species found either directly on, or in soils derived from, sandstone were

Table 1. Annotated pteridophyte species list for Carter Caves State Resort Park. An asterisk (*) indicates new report for CCSRP. Specimens from different herbaria are separated by a semicolon. Overall park abundances are indicated, followed by habitat preferences.

Aspleniaceae

**Asplenium montanum* Willd., Mountain Spleenwort – *Richardson 108* (MDKY); infrequent; sandstone cliff faces.

Asplenium pinnatifidum Nutt., Pinnatifid Spleenwort – *Richardson 28, 49, Meade 139, Huffaker 978* (MDKY); *Theiss s.n.* (KY); infrequent; sandstone cliff faces.

Asplenium platyneuron (L.) B.S.P., Ebony Spleenwort – *Richardson 39, Meade 138, Huffaker 179, Broomall 48* (MDKY); common; widespread.

**Asplenium resiliens* Kunze, Black-Stemmed Spleenwort – *Richardson 148* (MDKY); rare; limestone boulder.

Asplenium rhizophyllum L., Walking Fern – *Richardson 55, Huffaker 179, Ison 74, Meade 140, Mason 117, Huffaker 578* (MDKY); *Theiss s.n.* (KY); common; limestone cliffs and boulders.

Asplenium ruta-muraria L., Wall-Rue – *Richardson 147, Meade 133* (MDKY); *Terrell 1206, Weller s.n.* (KY); *Gilbert s.n.* (MUHW); infrequent; exposed dry limestone cliffs.

**Asplenium trichomanes* L., Maidenhair Spleenwort – *Richardson 109* (MDKY); rare; sandstone cliff bases.

Asplenium Xwherryi D. M. Smith et al., Wherry's Spleenwort – *Meade 152* (MDKY); assumed extirpated.

Blechnaceae

**Woodwardia areolata* (L.) T. Moore., Netted Chain Fern – *Richardson 342* (MDKY); rare; seep near sandstone cliff base.

Dennstaedtiaceae

**Dennstaedtia punctilobula* (Michx.) T. Moore, Hay Scented Fern - *Richardson 343, Risk 14086b* (MDKY); rare; sandstone cliff bases.

**Pteridium aquilinum* (L.) Kuhn var. *latiusculum* (Desv.) Underw. *ex* A. Heller, Bracken Fern – *Richardson 196.2* (MDKY); rare; soil below sandstone cliff.

Dryopteridaceae

Athyrium filix-femina (L.) Roth, Southern Lady Fern – *Richardson 24, 29, 35, Huffaker 979a* (MDKY); frequent; sandy soils.

Cystopteris bulbifera (L.) Bernhardi, Bulblet Bladder Fern – *Richardson 45, 54, 59, Meade 147, Levy 73* (MDKY); frequent; limestone cliff bases and limestone cobble dominated areas.

**Cystopteris protrusa* (Weath.) Blasdell, Southern Bladder Fern – *Richardson 60* (MDKY); infrequent; limestone cliffs and calcareous soils.

Cystopteris tennesseensis Shaver, Tennessee Bladder Fern – *Risk 14027, Lykens 290* (MDKY); rare; moist limestone cliffs and boulders.

Deparia acrostichoides (Sw.) M.Kato, Silvery Glade Fern – *Richardson 44, 47, Meade 151* (MDKY); infrequent; ravines and shaded slopes.

Diplazium pycnocarpon (Spreng.) M.Broun, Glade Fern – *Richardson 46, Ison 73, Huffaker 495b, Broomall 49* (MDKY); frequent; moist calcareous areas.

Dryopteris goldiana (Hook.) A.Gray, Goldie's Wood Fern – *Richardson 80, Meade 148* (MDKY); *Weller s.n.* (KY); rare; sandstone colluvium.

Dryopteris intermedia (Muhl. *ex* Willd.) A.Gray, Evergreen Wood Fern – *Richardson 52, 62, 63, Huffaker 176, Meade 135* (MDKY); *Theiss s.n.* (KY); common; sandstone cliffs and moist ravines.

Dryopteris marginalis (L.) A.Gray, Marginal Wood Fern – *Richardson 30, 38, Huffaker 175, Ison 72, Broomall 82* (MDKY); *Smith et al. 3449, 3450, Gilbert 871* (MUHW); common; moist ravines and slopes below sandstone cliffs.

Onoclea sensibilis L., Sensitive Fern – *Risk 14097, Huffaker 168, Weller s.n.* (MDKY); frequent; moist field edges.

Polystichum acrostichoides (Michx.) Schott., Christmas Fern – *Richardson 22, 26, 32, 33, Huffaker 600, King s.n., Meade 150* (MDKY); common; widespread.

**Woodsia obtusa* (Spreng.) Torr., Blunt-lobed Cliff Fern – *Richardson 214.2* (MDKY); *Gilbert 967* (MUHW); infrequent; limestone-dominated areas.

Equisetaceae

Equisetum arvense L., Field Horsetail – *Richardson 51* (MDKY); *Theiss s.n.* (KY); infrequent; riparian zones.

Equisetum hyemale L., Scouring Rush – *Richardson 57* (MDKY); *Meijer s.n., Theiss s.n.* (KY); *Trail 34, Watson 39* (MUHW); infrequent; riparian zones.

Hymenophyllaceae

**Trichomanes boschianum* Sturm, Appalachian Filmy Fern – *Richardson 110* (MDKY); rare; moist backwalls of sandstone rockhouses.

Isoetaceae

**Isoetes engelmannii* A. Braun., Engelmann's Quillwort – *Risk 14116a* (MDKY); rare; streamhead seeps.

Lycopodiaceae

Diphasiastrum digitatum (Dillenius *ex* A. Braun) Holub, Running Ground Cedar – *Richardson 53, Huffaker 171, Ison 76* (MDKY); *Theiss s.n., Meijer s.n.* (KY); infrequent; acidic soils.

**Huperzia lucidula* (Michx.) Trevis., Shining Firmoss – *Richardson 61* (MDKY); infrequent; sandstone cliffs and acidic soils.

Huperzia porophila (F.E.Lloyd & Underw.) Holub., Rock Clubmoss – *Richardson 25, Meade 134* (MDKY); infrequent; sandstone cliffs.

Table 1. Continued.

Lygodiaceae

**Lygodium palmatum* (Bernh.) Sw., Appalachian Climbing Fern – *Richardson 115* (MDKY); rare; sandstone cliff bases.

Ophioglossaceae

Botrychium biternatum (Savingy) Underw., Sparse-lobed Grapefern – *Meade 313* (MDKY); historical, unencountered during present study.

Botrychium dissectum Spreng., Dissected Grapefern – *Richardson 51, Meade 142* (MDKY); frequent; acidic soils below sandstone cliffs.

Botrychium virginianum (L.) Sw., Rattlesnake Fern – *Richardson 31.1, 36, Carr 52* (MDKY); *Warden s.n.* (KY); frequent; middle to lower slopes.

**Ophioglossum vulgatum* L., Adder's Tongue Fern – *Risk 13954, 14098* (MDKY); rare; riparian zone and moist slope.

Osmundaceae

Osmunda cinnamomea L., Cinnamon Fern – *Richardson 23, Meade 130* (MDKY); frequent; moist sandstone cliffs and acidic soils.

Osmunda claytoniana L., Interrupted Fern – *Richardson 40, 43, King 3701* (MDKY); frequent; moist sandstone cliffs and acidic soils.

Osmunda regalis L., Royal Fern – *Richardson 48, Meade 173* (MDKY); rare; streamhead seeps and wet sandstone cliff faces.

Polypodiaceae

Polypodium appalachianum Haufler & Windham, Appalachian Polypody – *Richardson 107* (MDKY); *Theiss s.n.* (KY); rare; sandstone cliff faces and boulders.

Polypodium virginianum L., Rock Cap Fern – *Richardson 31.2, 41, 56, 64, Broomall 47, Levy 72* (MDKY); frequent; sandstone cliffs and boulders.

Pteridaceae

Adiantum capillus-veneris L., Southern Maidenhair Fern - *G.G. Jr. s.n.* (GH); historical, presumed extirpated.

Adiantum pedatum L., Northern Maidenhair Fern – *Richardson 34, King s.n., Huffaker 186, 345, 580, Brown and Brown 10500* (MDKY); common; moist slopes and lowlands.

Pellaea atropurpurea (L.) Link, Purple Cliff Brake – *Richardson 67, Meade 139* (MDKY); *Theiss s.n.* (KY); frequent; limestone cliff faces.

Selaginellaceae

**Selaginella apoda* (L.) Spring., Meadow Spikemoss – *Richardson 75* (MDKY); infrequent; moist fields and lawns.

Thelypteridaceae

Phegopteris hexagonoptera (Michx.) Fée, Broad Beech Fern – *Richardson 37, 65, King 3710, Huffaker 595, Browne and Browne 10504* (MDKY); *McInteer 2518, 1198* (KY); common; moist soil.

Thelypteris noveboracensis (L.) Nieuwl., New York Fern – *Richardson 42, Huffaker 593* (MDKY); common; moist soil.

Vittariaceae

**Vittaria appalachiana* Farrar and Mickel, Appalachian Gametophyte Fern – *Risk 14575, 14952* (MDKY); rare; ceilings of sandstone rockhouses.

Asplenium montanum Willd., *A. pinnatifidum* Nutt., *A. trichomanes* L., *Dennstaedtia punctilobula* (Michx.) Moore, *Dryopteris goldiana* (Hook.) A. Gray, *D. intermedia* (Muhl. *ex* Willd.) A. Gray, *Huperzia porophila* (F. E. Lloyd and L. Underw.) Holub., *Trichomanes boschianum* Sturm, and *Vittaria appalachiana* Farrar and Mickel. Those pteridophytes with an exclusive relationship to limestone or its derived soils were *Asplenium resiliens* Kunze, *A. ruta-muraria* L., all three species of *Cystopteris*, *Diplazium pycnocarpon* (Spreng.) M. Broun, and *Pellaea atropurpurea* (L.) Link.

The formula for the species area curve was $S = 0.972A^{0.298}$ (where $S = \log_{10}$ number of species and $A = \log_{10}$ area in ha; $R^2 = 0.718$, $P < 0.001$) The predicted value of 21 for CCSRP based on this curve was less than half of the actual number, 47, of species and hybrids documented for the park (Figure 3).

DISCUSSION

Forty-six species and one hybrid representing 61% of Kentucky's total pteridophyte flora were documented from CCSRP. A large portion of the taxa known from Kentucky (Jones 2005) not documented from the park characteristically occur in wetlands (e.g., *Azolla caroliniana* Willd. and *Thelypteris palustris* Schott.) or on dry sandstone uplands (e.g., *Chelianthes lanosa* (Michx.) D. C. Eaton, *Diphasiastrum tristachyum* (Pursh) Holub, and *Lycopodium obscurum* L.). These two habitats are poorly represented within CCSRP. Species known from Carter or nearby counties and diligently searched for but not found in CCSRP were *Asplenium bradleyi* D. C. Eaton, *Cheilanthes lanosa*, *Diphasiastrum tristachyum*, *Dryopteris carthusiana* (Vill.) H. P. Fuchs, *Lycopodium*

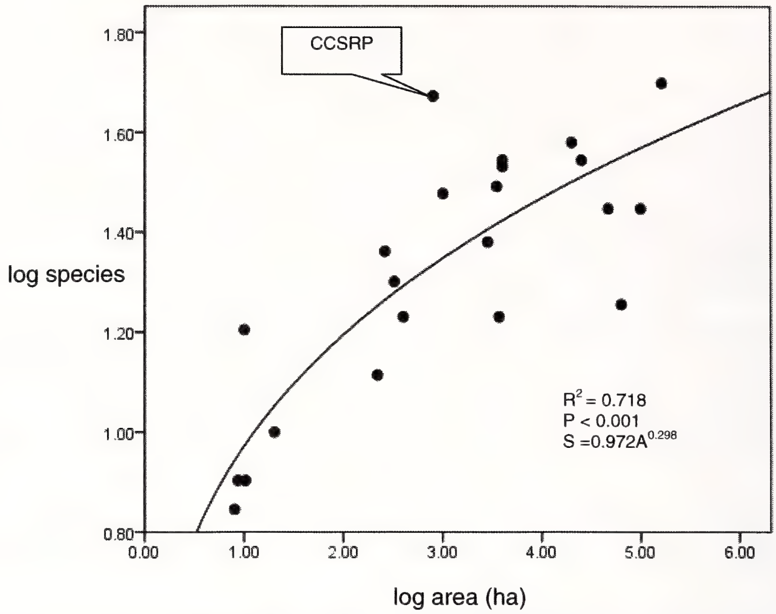

Figure 3. Species area curve for pteridophytes based on published floras with the data point for Carter Caves State Resort Park indicated. Studies included were Basinger et al. (1997); Blackwell et al. (1981); Bush (1988); Campbell and Meijer (1989); Carpenter and Chester (1987); Clements and Wofford (1991); Cranfill (1991); Davies (1955); Feeman (2002); Gaddy (1990); Grubbs and Fuller (1991); Hannan and Lassetter (1982); Ludwig (1999); Murrell and Wofford (1987); Palmer (1990); Schmalzer et al. (1985); Sole et al. (1983); Suiter and Evans (1999); Weckman et al. (2003); Wofford et al. (1979); Woods and Fuller (1988).

obscurum, and *Pleopeltis polypodioides* (L.) E. G. Andrews and Windham.

Based upon comparison with county record maps presented in Cranfill (1980) and Campbell and Medley (2006), *Cystopteris protrusa* (Weath.) Blasdell, *Isoetes engelmannii* A. Braun, *Ophioglossum vulgatum* L., and *Vittaria appalachiana* are new county records for Carter County.

Table 2. Floras with location, size, and index of similarity (IS) to Carter Caves State Resort Park. Index of similarity formula was IS= 2W/(A+B).

Study	Physiographic Province	Size (ha)	IS
Bush (1988)	Appalachian Plateau	8	0.222
Sole et al. (1983)	Appalachian Plateau	220	0.407
Weckman et al.(2003)	Appalachian Plateau	262	0.629
Wofford et al. (1979)	Appalachian Plateau	4000	0.716
Clements and Wofford (1991)	Appalachian Plateau	1000	0.727
Suiter and Evans (1999)	Appalachian Plateau	25,132	0.756
Schmalzer et al. (1985)	Appalachian Plateau	4000	0.790
Gaddy (1990)	Blue Ridge	10	0.444
Murrell and Wofford (1987)	Blue Ridge	2843	0.657
Basinger et al. (1997)	Coastal Plain	3702	0.413
Grubbs and Fuller (1991)	Coastal Plain	62,464	0.500
Woods and Fuller (1988)	Coastal Plain	99,460	0.639
Hannan and Lassetter (1982)	Interior Low Plateau	20.2	0.281
Blackwell et al. (1981)	Interior Low Plateau	10.3	0.296
Campbell and Meijer (1989)	Interior Low Plateau	400	0.469
Carpenter and Chester (1987)	Interior Low Plateau	325	0.545
Feeman (2002)	Interior Low Plateau	46,850	0.703
Davies (1955)	Interior Low Plateau	20,000	0.776
Cranfill (1991)	Interior Low Plateau	163,100	0.845
Palmer (1990)	Piedmont	3500	0.615
Ludwig (1999)	Valley and Ridge	8.7	0.291

Rare pteridophytes within the region collected during this study were Appalachian filmy fern (*Trichomanes boschianum*), Engelmann's quillwort (*Isoetes engelmannii*), and Goldie's wood fern (*Dryopteris goldiana*). Within the park there are numerous sandstone rockhouses. These create excellent habitat for the rare Appalachian filmy fern (*Trichomanes boschianum*). Four rockhouses within the Horn Hollow region of the park contained populations of the Appalachian filmy fern with the largest population covering approximately 2.79 m^2 of substrate. Two populations of Engelmann's quillwort (*Isoetes engelmannii*) were found in the Cascade Caves region of the park. These two populations were in slightly swampy streamheads that contained abundant cinnamon fern (*Osmunda cinnamomea* L.), royal fern (*Osmunda regalis* L.), and New York fern (*Thelypteris noveboracensis* (L.) Nieuwl). It is hypothesized that Engelmann's quillwort may be more common in eastern Kentucky than current collections indicate due to its habitat, atypical pteridophyte morphology, and relatively small size. Goldie's wood fern (*Dryopteris goldiana*) was found along a heavily used trail just uphill from the entrance to Saltpetre Cave and along the sandstone cliff line southeast of the Saltpetre Cave site.

Pteridophytes that were rare within CCSRP were *Asplenium resiliens* (one site, but with well developed large plants), *Asplenium trichomanes* (several individuals in one location), *Dennstaedtia punctilobula* (two sites), *Dryopteris goldiana* (three sites along one cliff), *Isoetes engelmannii* (two sites), *Lygodium palmatum* (Bernh.) Sw. (two small plants at one site), *Ophioglossum vulgatum* (two sites), *Osmunda regalis* (three sites), *Pteridium aquilinum* (L.) Kuhn (one site), *Trichomanes boschianum* (four sites along one cliff line), *Vittaria appalachiana* (two sites), and *Woodwardia areolata* (L.) T. Moore (one site). Habitat destruction could be related to the rarity of *Dryopteris goldiana* and *Pteridium aquilinum* in CCSRP. The Goldie's Wood Fern population is bisected by a heavily used trail and the only Bracken Fern site was beside another heavily used trail within 100 m of the lodge. Habitat specificity may account for the rarity of some species, e.g., *Isoetes engelmannii*, *Ophioglossum vulgatum*, *Osmunda regalis*, *Trichomanes boschianum*, *Vittaria appalachiana*, and *Woodwardia areolata*. These ferns, with the exception of *T. boschianum* and *V. appalachiana* that inhabit deep sandstone overhangs, require very moist to almost swamp-like conditions, a relatively uncommon habitat within CCSRP.

Adiantum capillus-veneris was collected from the Carter Caves region in 1899. This specimen represents an important collection because this is very near the northern limit of its geographical range. However, it is not certain whether the species occurred within the current park boundaries because at the time of the collection the park was not publicly owned and did not have the same boundaries as it does today. Also, the location data on the label is typical for collections of this time period and was not very precise. Because the locality data on the label is vague, the exact site for collection of this specimen is not known. The researchers diligently looked in all appropriate habitats for an extant population of this fern, but in the sites examined none were found. One potential location of suitable habitat was not examined because the site was an inaccessible ledge in the midslope of a large cliff. Another possibility is that the *A. capillus-veneris* may have been in a location since submerged by the reservoir on Smokey Creek. For the purposes of this study, it is assumed that this species did occur, at least historically, within the present boundaries of the park. This species is presumed extirpated from within the park.

A specimen of *Asplenium Xwherryi* D. M. Smith *et al.* collected at CCSRP is housed at MDKY. This hybrid is presumed to be extirpated from CCSRP. Examination of the locality data on the label, in conjunction with interviewing a retired park employee, allowed the researchers to relatively confidently pinpoint the former population's location. This population was in a relatively high traffic area, and general human disturbances could have been the cause of its extirpation. Also, excessive collecting may have extirpated this hybrid, since there are several duplicates of this hybrid housed at MDKY.

The index of similarity (IS) values between the pteridophyte floras of CCSRP and other areas (Table 2) were fairly high. This was expected because most pteridophyte species

have large geographical ranges. The highest index of similarity value was between CCSRP and Hardin County, Kentucky (Cranfill 1991), at 0.845. This is notable because this study occurred in a different physiographic region of the state, the Interior Low Plateau, than that within which CCSRP is found. Hardin County does, however, contain both sandstone and limestone substrate. The area with the lowest IS with CCSRP was a wet meadow in Barbour County, West Virginia (Bush 1988), at 0.222. This study occurred in the same physiographic province as the current study. It was, however, a very small (8 ha) and specialized habitat that likely accounts for this large disparity.

CCSRP lies well above the expected value for an area of its size, being further above the line than any other flora included for comparison (Figure 3). Some reasons for this high species richness are the presence of many sandstone and limestone outcrops within the park, the relatively low amount of disturbance that has occurred to the forests, and the geographic location of CCSRP.

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Proterometra macrostoma (Faust) (Trematoda: Azygiidae): Further Studies on Strains at North Elkhorn Creek, Scott County, Kentucky

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ABSTRACT

The objectives of the present study were (1) to compare the frequency of *Proterometra macrostoma* (Faust) strains and the number of single vs. multiple strain infections in snails, *Elimia semicarinata*, collected from North Elkhorn Creek during June 2000, 2004, and 2007, (2) to measure selected cercarial strain egg loads and swimming distance under red and white light, and (3) to determine the developmental rate (based on egg stages) of strain I and III adult worms in the bluegill, *Lepomis macrochirus*. The frequencies of strains I (11.1–28.6%) and III (21.3–35.7%) were the highest, while strains II (4.2–8.8%) and VI (0–1.4%) have been relatively uncommon at North Elkhorn Creek over the last seven years. The majority (76.2–94.3%) of infected snails had multiple strain infections. No significant difference was found between the average egg number (strains I, II, III, IV, V, VII, and VIII) or mean swimming distance (strains I, III, and VIII) of cercariae under red or white light. Similarly, no significant difference was found between the average number of stage I, II, and III eggs, respectively, found in strain I and III adult worms on days 12 and 20 postinfection in experimentally infected bluegill.

KEY WORDS: *Proterometra macrostoma*, cercariae, strains, Kentucky, North Elkhorn Creek

INTRODUCTION

Proterometra macrostoma (Faust) is a digenetic trematode found in streams and rivers east of the Mississippi River. The life cycle incorporates a snail intermediate host and a centrarchid fish definitive host. The latter becomes infected upon ingestion of the parasite's unusually large (3.0–9.0 mm) progenetic cercaria whose swimming is marked by vertical bursts (36.0–150.0 mm) in the water column. The adult worm, once ingested, exits from its cercarial tail and attaches to the esophagus and stomach of the fish host.

Horsfall (1934), in her study of *Cercaria macrostoma* (=*P. macrostoma*) from snails collected at Homer Park, Illinois, the Des Plaines River, Illinois, and the Oconomowoc River, Wisconsin, noted that the number, size, and position of papillae or mammalations varied considerably in fresh specimens of the worm. Dickerman (1945) reported three distinct cercarial types of *P. macrostoma* in snails obtained from the Bass Island region of western Lake Erie based on color, the distribution of mammalations with and without spines, morphological measurements, and the presence/absence of eggs. Initially he thought that these "types" represented three separate species, but experimental infections of fish resulted in the recovery of just a single type of adult worm. Dickerman (1945) concluded that his cercarial types were in fact varieties that had not evolved to the point of being considered as separate species. Riley and Uglem (1995) attempted to sort out whether Dickerman's (1945) cercarial variations, "represent phenotypic plasticity, strains or distinct species." Working primarily at North Elkhorn Creek, Kentucky, Riley and Uglem (1995) separated this species into eight strains based on the following cercarial features: (1) distribution of papillae with and without spines, (2) daily and seasonal emergence patterns, (3) initiation and distance/duration of swimming under red light, and (4) degree of infectivity in different species of centrarchid fish. They also determined the relative frequency of these *P. macrostoma* strains at North Elkhorn Creek during May 1990 and 1991.

The continued presence and frequency of these eight strains in the *Elimia semicarinata* (Say) snail population at North Elkhorn Creek since Riley and Uglem's (1995) original study in 1990 and 1991 has not been evaluated.

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Because possible changes in these strains may be reflective of changes in the preferred centrarchid host community at the site (Riley and Uglem 1995), the purpose of the present study was to summarize the frequency of these strains and the number of single vs. multiple strain infections in snails collected from North Elkhorn Creek during June 2000, 2004, and 2007. In addition, an attempt was made to further characterize selected strains of this parasite by comparing immature egg loads in cercariae, cercarial swimming distance under red and white light, and developmental rate in the fish definitive host based on stage of egg maturity.

MATERIALS AND METHODS

For all experiments, *E. semicarinata* were collected from North Elkhorn Creek (lat 38°11′00″N, long 84°29′19″W), Scott County, Kentucky, during June 2000, 2004, and 2007 and screened for mature cercarial infections with *P. macrostoma*. The latter was accomplished by placing snails in an environmental chamber at 20°C and a 12/12 hr light/dark cycle for three consecutive days and monitoring the emergence of cercariae. Infected snails were then individually placed in isolated chambers within compartmentalized boxes.

Individual snails were assigned numbers to ensure proper tracking during the 21-day experiment, and maintained at 20°C in a 12/12 hr light/dark cycle. Cercarial emergence from each snail was monitored daily, each freshly emerged cercaria identified to strain according to Riley and Uglem (1995), and the number of single and multiple strain infections recorded at the termination of the experiment. Egg counts were also made from some of these cercariae. ANOVA was used to assess possible differences between the mean egg loads of cercarial strains with adequate sample sizes.

Previous work had shown significant differences in *P. macrostoma* cercariae swimming distances under red vs. white light (Rosen et al. 2005a). To assess possible differences in cercarial swimming according to strain in these two light regimens, 2-L graduated cylinders were filled with artificial pond water (APW; 0.5 mM NaCl, 0.05 mM KCl, 0.4 mM $CaCl_2$ and 0.025 mM $MgCl_2$). They were then placed under broad spectrum 20-watt fluorescent lights wrapped in black fiberglass window screening or red filters (Edmund Scientific) and adjusted to achieve an intensity of 11 foot candles (fc; Extech Light Meter) at the top of each cylinder. This intensity approximated daylight field values (14.5 fc) at North Elkhorn Creek at 0.6 m depth. One cercaria (less than 2 hr post-emergence) was then placed into a cylinder and exposed in 7-min intervals to red and then white light. Each 7-min time span consisted of a 2-min acclimation period followed by a 5-minute recording period. A single swimming burst distance was recorded at the beginning of each minute of the 5-min recording interval for each light treatment, and the average distance (using a conversion of 1.0 ml = 0.2 mm) was calculated from these five observations. At the termination of each swimming experiment, the cercaria was recovered and its strain determined using papillae and spine distribution. ANOVA was used to assess possible differences between the mean swimming distances of strains I, III, and VIII under both red and white light.

Due to the progenetic nature of the *P. macrostoma* cercaria, it is only possible to measure developmental rate of this worm in the definitive host by the maturity of its egg stages (Rosen et al. 2000, 2005b). To evaluate possible differences in the developmental rate of strain I and III adults in the bluegill, *Lepomis macrochirus* Rafinesque young (40–80 mm) fish were obtained from Pfeiffer Fish Hatchery in Frankfort, Kentucky. They were placed in 37.85-L tanks at 22.7 ± 4.6°C and maintained on fish pellets acquired from the hatchery. Individual fish were removed from their tanks and placed in smaller, 3.79-L tanks for infection with 1–3 cercariae previously identified to strain I or III with the aid of a compound microscope. Once all introduced cercariae were ingested (generally less than 1-hr), the fish were returned to their respective holding tanks. Fish were anesthetized in MS-222 (tricaine methane sulfonate) and necropsied on days 12 and 20 PI (postinfection). The eggs in the recovered worms were counted and categorized as either stage I (clear cell at opercular end and dark vitelline mass at opposite end), stage II (vitelline mass restricted to periphery; advanced cleavage), or stage III (dark yellow shell; fully-formed miracidi-

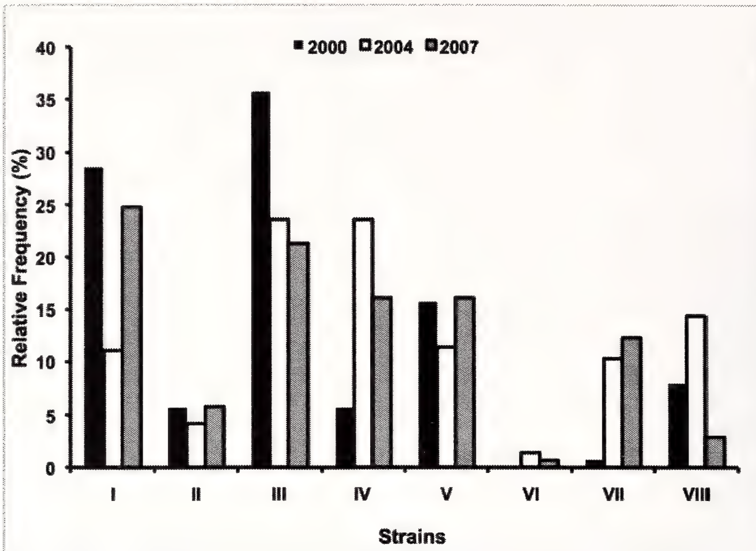

Figure 1. Relative frequency of eight *Proterometra macrostoma* cercarial strains at North Elkhorn Creek in June 2000 (N=140 cercariae), 2004 (N=364 cercariae), and 2007 (N=310 cercariae).

um) according to Rosen et al. (2000, 2005b). A Student's *t*-test was used to determine possible differences in the mean number of each of the egg stages present on days 12 and 20 PI, respectively, between these strains. A probability of $P \leq 0.05$ was considered as significant for all statistical tests.

RESULTS

Strains I and III were the most prevalent, while strains II and VI were relatively uncommon during June 2000, 2004, and 2007 (Figure 1). Only a small fraction of snails had single strain infections; most had multiple strain infections (2–5), with two strains being the most common (Figure 2). In snails possessing single or multiple infections, there was no obvious pattern regarding the release of the different cercarial strains over the 21-day observation period (Figures 3a–3d).

The number of eggs within the various cercarial strains varied considerably (i.e., range 0–40), but no significant difference ($F = 1.869$: df = 6, 436; $P = 0.085$) was found in the average egg numbers between cercarial strains (Figure 4). Similarly, no significant difference was found between the mean swimming distances of strain I, III, and VIII cercariae under red ($F = 2.183$: df = 2, 83; $P = 0.961$) or white ($F = 2.621$: df = 2, 83; $P = 0.079$) light (Figure 5).

No significant difference was found between the average number of stage I ($t = 1.287$; df = 29; $P = 0.208$) or stage II ($t = 0.369$; df = 29; $P = 0.714$) eggs in strain I and III worms on day 12 PI (Figure 6). Similarly, no significant difference was found between the average number of stage I ($t = 1.875$; df = 16; $P = 0.079$), stage II ($t = 0.943$; df = 16; $P = 0.360$), or stage III ($t = 0.599$; df = 16; $P = 0.558$) eggs in strain I and III worms on day 20 PI (Figure 6).

DISCUSSION

According to Bryant and Flockhart (1986), there is little chance of population divergence/speciation when gene exchange is high; conversely, when gene exchange is low, there is a good chance for speciation. Somewhere between these extremes the populations become sufficiently different to be considered as variants or strains (Bryant and Flockhart 1986). Such "moderate" gene flow in the *P. macrostoma* population at North Elkhorn Creek seems to have been relatively unchanged over the last 17 yr as reflected by the strain types present and their comparatively consistent frequencies. In the present study, the highest frequencies were observed

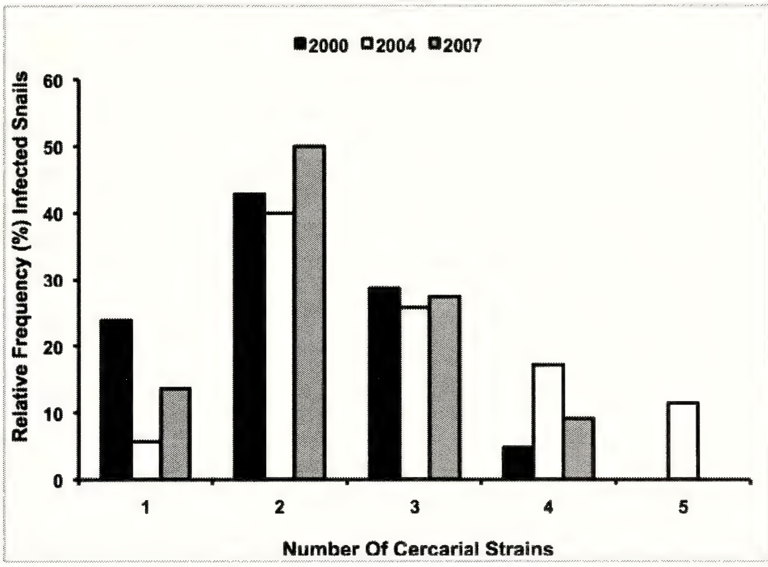

Figure 2. Relative frequency of single vs. multiple *Proterometra macrostoma* strain infections in *Elimia semicarinata* at North Elkhorn Creek in June 2000 (N=21 snails), 2004 (N=35 snails), and 2007 (N=22 snails).

for strains I and III, while strains II and VI have been relatively uncommon between 2000–2007. Similarly, Riley and Uglem (1995) also found that strains I and III were the dominant forms of *P. macrostoma* at their two Kentucky sites (North Elkhorn Creek and Cane Run Creek) in 1990/1991. However, their evaluation of infected snails from the

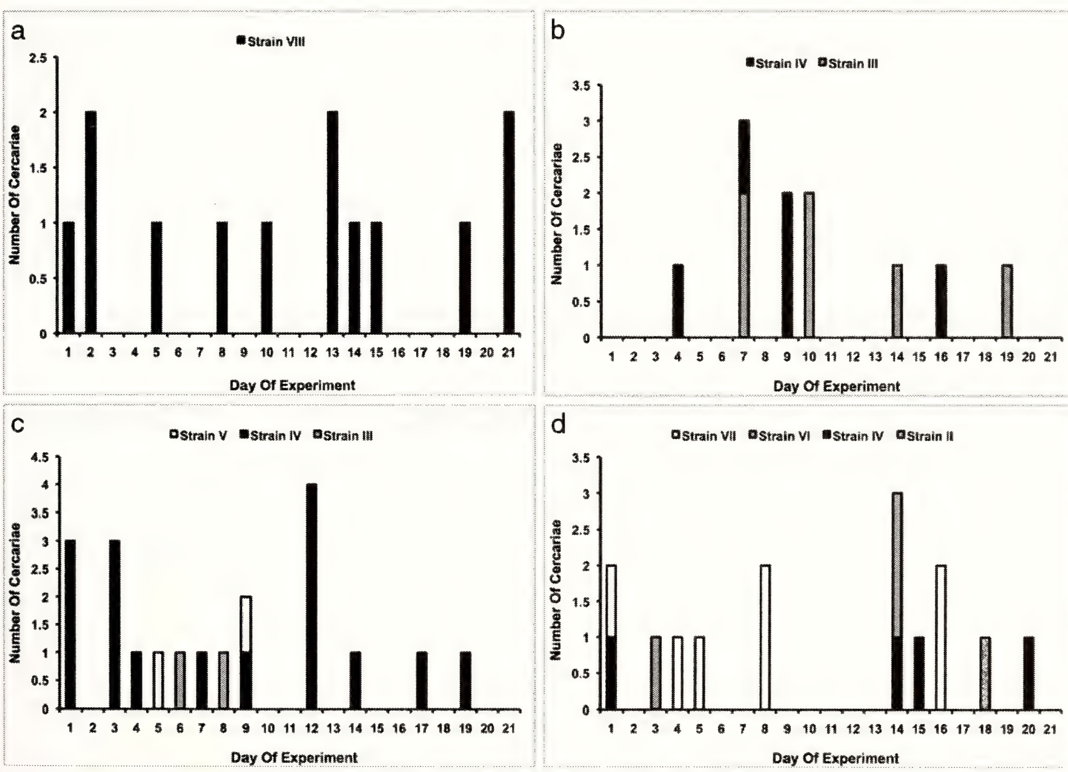

Figure 3. Examples of cercarial shedding patterns over 21 days from individual snails, *Elimia semicarinata*, infected with 1–4 strains of *Proterometra macrostoma*. (3a=one strain; 3b=two strains; 3c=three strains; 3d=four strains).

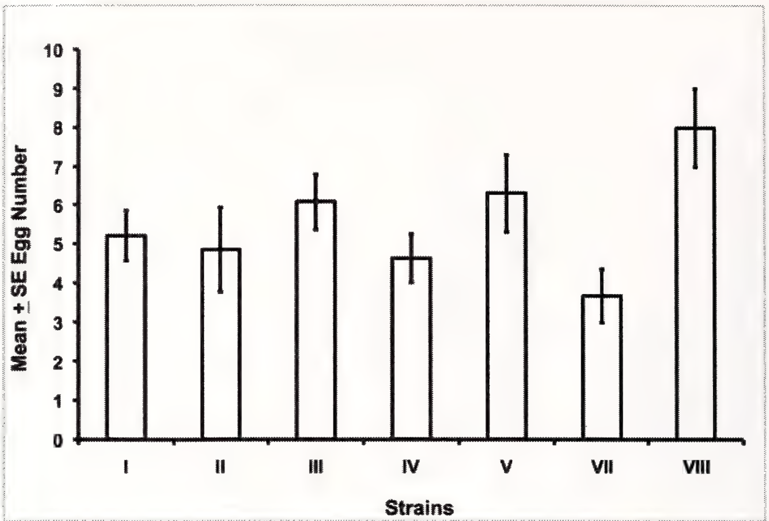

Figure 4. Mean ± SE eggs / cercarial strain of *Proterometra macrostoma*. (Number of cercariae evaluated: I=93; II=26; III=97; IV=87; V=60; VII=46; VIII=35).

Olentangy River, Ohio, and Carp Lake River, Michigan, showed a notable decrease in strain I when compared with their Kentucky locales. Based on experimental infections of several centrarchid species, Riley and Uglem (1995) concluded that strains of *P. macrostoma* have preferred centrarchid hosts, and that when the prevalence of a preferred host changes, so does the frequency of its associated strain. For example, Riley and Uglem (2005) linked the high frequency of strain I in Kentucky to the high prevalence of its preferred host, *Lepomis gulosus* (Cuvier), the warmouth bass. By contrast, the decrease of this strain's frequency in Ohio and Michigan was associated with the absence of this host species. Because the dominance of strain I has remained relatively stable at North Elkhorn during 1990/1991, 2000, 2004, and 2007, it can perhaps be concluded that the frequency of the associated warmouth population at this site has remained unchanged as well. Riley and Uglem (2005)

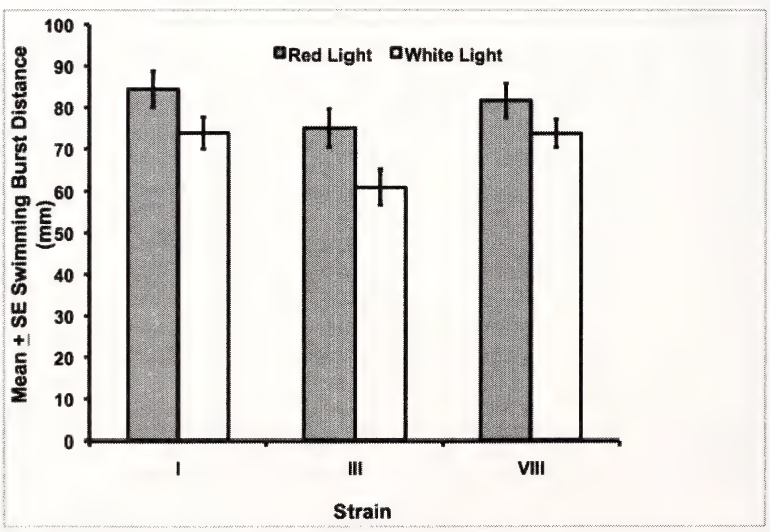

Figure 5. Mean ± SE vertical swimming burst distance of three different *Proterometra macrostoma* cercarial strains under red and white light. (Number of cercariae evaluated: I=45; III=20; VIII=21).

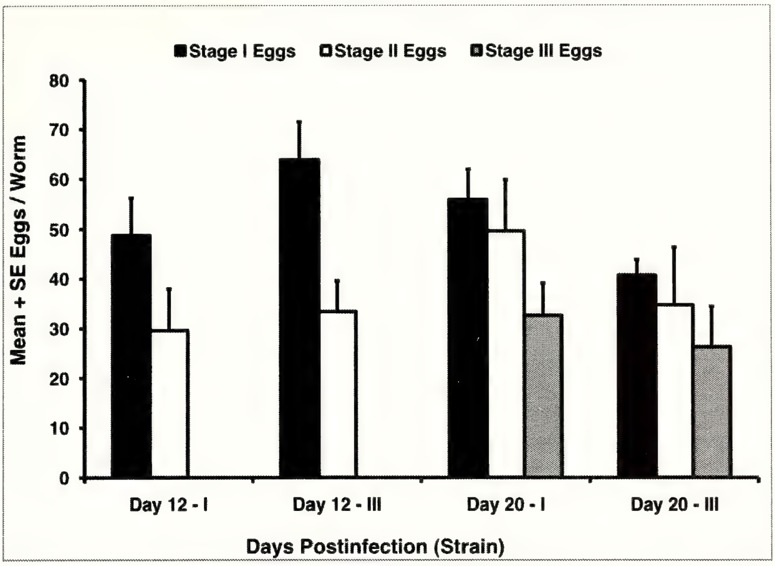

Figure 6. Mean + SE egg stages in *Proterometra macrostoma* strain I and III adults on days 12 and 20 postinfection in experimentally infected bluegill at 22.7°C. (Number of worms evaluated: Day 12 (I)=11; Day 12 (III)=20; Day 20 (I)=11; Day 20 (III)=7).

also suggested that strains with consistently low frequencies (e.g., strains II and VI in this study) might be the result of natural crossings of strains. Such crossbreeding between digenean strains has been particularly well-documented for the schistosomes. Theron (1984) described two strains of *Schistosoma mansoni* Sambon based on cercarial shedding patterns. The strain shedding during the day utilized humans as their primary definitive host while the strain that shed during the night utilized a rat definitive host. When Theron and Combes (1988) crossed these two strains, the resulting F_1 had an intermediate shedding pattern demonstrating the genetic basis for this phenomenon.

Due to the nature of asexual polyembryony of *P. macrostoma* in its snail intermediate host, the cercariae produced are clones of the original egg ingested by the snail. Thus a snail shedding only one cercarial strain is presumed to have only been infected with one or more egg(s) of the same strain. Riley and Uglem (2005) found that 66% of the snails they monitored over two weeks had pure (single) strain infections. They indicated that this high proportion of pure infections was related to the fact that most snails become infected with only one egg. By contrast, only 5.7–23.8% of the snails monitored for three weeks in our study had single strain infections. Though individual snails can contain a number of *P. macrostoma* rediae asexually producing cercariae, only 0.250–0.833 cercariae/snail/day are shed in sample snail populations (Rosen et al. 2005c). The extended time allowed for cercarial development and emergence (i.e., three vs. two weeks) in our study may have been, in part, responsible for an increase in the number of multiple strain infections detected (i.e., our additional week of observation may have provided more time for cercariae from a different egg and possibly strain to develop and be shed).

Although further attempts were made to "biologically" characterize several selected strains of *P. macrostoma*, no new differences were noted. No significant difference was found in the average number of eggs in the seven cercarial strains we evaluated. By contrast, Dickerman (1945) indicated that his type I cercaria had 30–50 eggs, but types II and III never contained eggs. Riley and Uglem (1995) indicated that Dickerman's (1945) three types correlated with their strains I, IV and VIII, respectively. While strains IV and VIII occasionally lacked eggs in our study, the majority had a number of developing eggs. We also found no significant difference in the vertical swimming distances of strains I, III,

and VIII under red or white light, and all cercarial swimming bursts were initiated at the bottom of the cylinder. By contrast, Riley and Uglem (1995) found that while the swimming burst distance was similar between strains III and VIII under red light, the bursts were initiated at significantly different heights in the water column. We have no explanation for this discrepancy, though, in our experiments, it appeared that tactile stimulation of the cercarial tail by the bottom of the cylinder served to initiate the swimming burst. Finally, Riley and Uglem (1995) found that strain I cercariae established much better in bluegill than strain III worms based on their experimental infections. However, the subsequent developmental rate (based on the average number of stage I, II, and III eggs) of strain III in bluegill in the present study was not significantly different from strain I at 12 and 20 days PI. This result demonstrated a lack of connection between the initial infectivity of *P. macrostoma* and its subsequent developmental rate as a strain characteristic in the definitive host.

Future work will focus on experimental infections of snails and fish with known strains of this worm to determine whether or not these proposed strains are "true-breeding." Such studies will also make it possible to conduct breeding experiments between strains provided that cross-fertilization between adult worms takes place in the fish definitive host. In addition, we will continue our work to characterize genetic patterns in selected strains of *P. macrostoma* using RAPD (random amplification of polymorphic DNA).

ACKNOWLEDGMENTS

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Diatom Species Composition and Environmental Conditions at Four Perennial Springs in Western Kentucky and Tennessee

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ABSTRACT

Diatom assemblage composition and water chemistry at four springs of different geological origin in western Kentucky and Tennessee (Land-Between-the-Lakes National Recreation Area, LBL) were examined using unglazed quarry tiles as artificial substrates. Significant differences in diatom species composition and water chemistry were found among the four springs. A total of 25 taxa were identified with 9 taxa being common to all springs. Calciphilous *Planothidium lanceolata* var. *lanceolata* (Bréb.) Grunow and *Cocconeis placentula* var. *lineata* (Ehr.) V. H. were the dominant species in the carbonate streams of limestone geology (Mint and Panther springs). Acidophilous *Eunotia intermedia* (Krasske ex. Hust), *Achnanthidium minutissimum* var. *saprophila* Kobaysi and Mayama, and *Fragilariforma virescens* (Ralfs) Williams and Round were most abundant in the streams with siliceous and argillaceous geology (Barnett and Brown Springs). Statistical analyses indicated that diatom species composition was most highly correlated with conductivity and suggested that the underlying geology of the springs played a role in periphyton community composition.

KEY WORDS: Diatoms, water chemistry, springs, geology, Land-Between-the-Lakes, Kentucky, Tennessee

INTRODUCTION

Diatoms are important in aquatic ecosystems because they influence all higher trophic levels and are known for their sensitivity to chemical conditions (Lowe 1974); therefore, they are a useful supplement to chemical analyses in assessments of water quality and ecosystem health. Leira and Sabater (2005) found that freshwater diatoms have affinities towards certain ions. A number of taxa have been characterized as preferring calcium-rich or calcium-poor waters. For example, some *Cymbella* species are calciphilous, some *Diatoma* are halophilous, while some *Eunotia* are acidophilous (Potapova and Charles 2003). Further, the optimal conductivity for many species of *Achnanthes*, *Cocconeis*, and *Gomphonema* is relatively high compared with species of *Eunotia*, *Diatoma*, and *Navicula*. Other studies have shown that some of these same taxa having high optimal conductivity requirements also have optimal tolerances to high pH levels. Many taxa with low optimal conductivity requirements also have low optimal pH levels (Pan et al. 1996). Requirements for nutrients also differ among diatom taxa at both the generic and specific levels (Leira and Sabater 2005).

Because underlying parent geology is a regional influence on the chemical composition of groundwater, geology should be considered when evaluating diatom assemblages or periphyton of flowing water, particularly springs and headwater streams (Pan et al. 1996; Leland and Porter 2000; Leira and Sabater 2005). Even in fairly uniform geologic regions, groundwater characteristics may differ over small distances, depending on the locations of recharge zones, the lengths of underflow paths, and the ages of the water sources. Even small differences in conductivity, alkalinity, pH, or nutrient concentrations may be reflected in periphyton colonization rates and species assemblages (Leland and Porter 2000; Rimet et al. 2004).

Only limited information exists on periphyton distributions for western Kentucky streams (Camburn 1982; Hendricks et al. 2006), Lake Barkley (Jarrett and King 1989), or Kentucky Lake (Barnese 1984), while virtually no information exists on the water chemistry or periphyton of Land-Between-the-Lakes National Recreation Area streams (LBL). The purposes of this study were to provide a first assessment of the diatom species present in four perennial springs at LBL using artificial substrates and to determine the relationships between physicochemical parameters and diatom species compositions.

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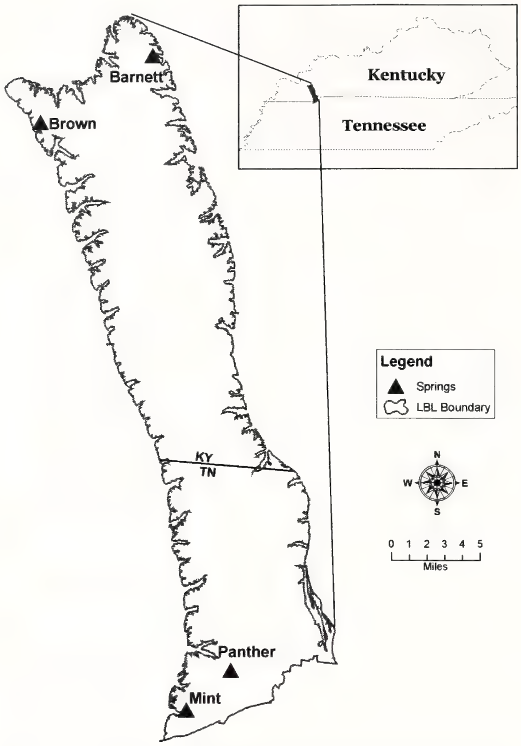

Figure 1. Map of study spring at Land-Between-the-Lakes National Recreation Area, western Kentucky and Tennessee.

STUDY AREA

The springs were located in the Land-Between-the-Lakes National Recreation Area (LBL) managed by the U.S. Forest Service. Two springs were in northern Lyon Co., Kentucky (Brown Spring; 36°57.363′N, 88°11.351′W, and Barnett Spring; 37°00.797′N, 88°04.529′W) and two in Stewart County, Tennessee (Panther Spring; 36°30.373′N, 87°58.857′W, and Mint Spring; 36°28.360′N, 88°01.501′W) (Figure 1). The four springs are perennial with uniform flow year around and were well known to the moonshiners of the Prohibition Era for their excellent taste quality (Mauer 1974). The springs are in mature oak-hickory forests and are heavily canopied.

Panther and Mint Springs are geologically similar overlying alluvium, Warsaw Limestone and the Fort Payne Formation (USGS Geologic Quadrangles: Hamlin and Paris Landing GQ, 1966; Tharpe TN GQ, 1967). The alluvium is composed of silt and clay and in places is stratified with lenses of sand and gravel. Thickness ranges from 0–12 m. Warsaw Limestone is composed of tightly packed fine to medium fossil fragments, is interbedded with microcrystalline shale and chert limestone, and weathers to silty clay and blocky chert composed of silicified fossil grains or rounded dense nodular chert. Thickness ranges from 41–47 m. The Fort Payne Formation is composed of 10–20% chert and silty limestone that is hard and thin-bedded. The rock weathers deeply to crumpled layers of residual chert, silt, and tripolitic (diatomaceous earth) clay. The thickness usually is >91 m.

Barnett Spring directly overlies the alluvium (USGS Geologic Quadrangle: Eddyville KY GQ, 1963). Immediately surrounding the alluvium is the Tuscaloosa Formation, a gravel mixture that is sandy, silty, and in some places argillaceous (containing clay). The rock formations are thin-bedded with scattered lenses of chert, sand, and tripolitic (diatomaceous earth) silt or clay. Thickness ranges from 0–53 m.

Brown Spring overlies the Tuscaloosa Formation and sand (USGS Geologic Quadrangle: Birmingham Point KY GQ, 1966). The sand is slightly argillaceous, silty, and unconsolidated in places. The sand is composed of poorly sorted medium–fine sand grains, of which 85%–95% are quartz, 5%–15% are chert, and ≤2% are mica or other minerals.

METHODS

Unglazed ceramic quarry tiles (area = 91 cm^2) (Wellnitz et al. 1996) were placed downstream from each spring in October 2006. Tiles were allowed to colonize with periphyton for approximately four weeks and then were collected in triplicate from each spring every four weeks until July 2007. Tiles were placed as closely as possible to the spring outlet. The tiles at Mint and Panther springs were placed ~8–10 m from their sources, while tiles at Barnett and Brown springs were placed ~3–5 m from their sources. Tiles were collected, put in Zip-Loc® bags, and placed in a cooler with ice for transport to the laboratory for analysis.

In the laboratory, periphyton was scraped off each tile with a toothbrush into a beaker. The diatoms were then cleared with a mixture of 30% H_2O_2 (hydrogen peroxide) and $K_2Cr_2O_7$ (potassium dichromate), followed

by several rinses using deionized water (Carr et al. 1986). Permanent slides were mounted using Naphrax. Identifications were made under a light microscope (Zeiss Axioplan, Germany) using 1000× magnification and oil emersion (Eaton et al. 2005). A minimum of 200 diatom valves were counted on several transects across each slide surface, and species were identified using standard taxonomic keys (Patrick and Reimer 1966; Krammer and Lange-Bertalot 1986, 1988, and 1991).

Water temperature, pH, conductivity, dissolved oxygen (DO), and turbidity were measured in triplicate in the vicinity of the tiles during each diatom sampling period with a YSI multi-probe meter (Yellow Springs Instruments, Yellow Springs, OH). Triplicate water samples from each site were collected and analyzed in the laboratory for $NH4^+$, NO_3^-+NO_2^-, SiO_2, PO_4^{3-}, Cl^-, and SO_4^{2-} using a Latchet Autoanalyzer (Milwaukee, WI) and standard reagents (Prokopy and Switala 1994). Alkalinity was determined using single-point titration methods and formula of Eaton et al. (2005). Light at the stream surface at mid-day was measured at each site with a LI-COR model 185B photometer. Discharge was determined at each site using a Marsh McBirney flow meter and cross sectional area measurements of the stream.

Analysis of variance (ANOVA) was performed to determine if there were any significant differences in physicochemical and nutrient variables among the four springs. The Tukey Kramer multiple comparison procedure was used to identify where the differences occurred. Level of significance was set at $\alpha = 0.05$.

Cluster analysis was used to determine the grouping structure of the springs based on similarities in their physicochemical and nutrient variables and taxa (either genus only or genus and species) composition and to give a visual representation of how similar the taxa composition and physicochemical variables were at each spring.

A principal component analysis (PCA) was used to reduce physicochemical data dimensions from 13 variables to the most relevant subset based on their eigenvalues. Using SYNTAX 2000, a canonical correlation analysis (CCA) was then performed using the reduced data from the PCA results to determine the strength of the relationships between the taxa composition and environmental variables at each spring.

RESULTS

Different diatom assemblages (Table 1) and relative abundances (Figure 2) characterized each of the four springs with Mint and Panther Springs being most similar. Dominant at Mint Spring were *Cocconeis placentula* var. *lineata* (74.3% of the total valves counted), *Planothidium lanceolata* var. *lanceolata* (17.5%), *Gomphonema minutum* (4.6%), and *Eunotia intermedia* (1.9%). Dominant at Panther Spring were *Planothidium lanceolata* var. *lanceolata* (56.4%), *Cocconeis placentula* var. *lineata* (22.3%), *Gomphonema minutum* (18.42%), and *Eunotia intermedia* (1.0%). The most abundant species at Barnett Spring were *Eunotia intermedia* (56.6%), *Fragilariforma virescens* (28.1%), *Gomphonema parvulum* (5.07%), *Pinnularia gibba* (3.5%), *Planothidium lanceolata* var. *lanceolata* (2.8%), and *Gomphonema minutum* (1.5%), while the most abundant species at Brown Spring were *Eunotia intermedia* (52.8%), *Achnanthidium minutissima* var. *saprophila* (24.6%), *Meridion circulare* var. *circulare* (8.0%), *Fragilarforma virescens* (5.4%), *Gomphonema parvulum* (2.7%), and *Planothidium lanceolata* var. *lanceolata* (2.2%).

There were no significant differences in light availability among sites although Panther Spring appeared to have less canopy cover at mid-day ($P \geq 0.05$) (Table 2). Discharge was higher at Mint and Panther Springs (0.018 and 0.022 m^3 sec^{-1}) compared with the smaller Barnett and Brown Springs (0.001 and 0.002 m^3 sec^{-1}). Conductivity and dissolved oxygen (DO) were significantly different among all four sites. Mint and Panther Springs had significantly higher average conductivity (205.4 and 250.8 µS cm^{-1}, respectively) compared with Barnett and Brown Springs (34.2 and 53.5 µS cm^{-1}, respectively). Water temperatures at each spring did not vary greatly throughout the sampling period with the ranges of <2 °C among the springs. Panther and Barnett Springs both had average temperatures of 14.2 °C. Mint Spring averaged 14.8 °C and Brown Spring averaged 13.1°C. Average DO at Mint and Panther Springs (7.1 and 7.9 mg L^{-1}, respectively) was significantly

Table 1. Checklist of diatom taxa found in Mint, Panther, Barnett, and Brown Springs in western Kentucky and Tennessee (Land-Between-the-Lakes National Recreation Area).

Taxon	Mint	Panther	Barnett	Brown
Achnanthidium minutissimum var. *saprophila* Kobaysi & Mayama	X	X	X	X
Amphora perpusilla (Grun.) Grun	X			
Caloneis hyaline Hust.				X
Cocconeis placentula var. *lineata* (Ehr.) V.H.	X	X	X	X
Cymbella species of unknown or uncertain identity			X	X
Eunotia intermedia (Krasske ex. Hust) Nörpel & Lange-Bertalot	X	X	X	X
Eunotia paludosa Grun			X	X
Eunotia species of unknown or uncertain identity				X
Fragilariforma virescens (Ralfs) Williams & Round	X	X	X	X
Frustulia species of unknown or uncertain identity			X	X
Gomphonema acuminatum Ehr.			X	
Gomphonema minutum (Agardh) Agardh	X	X	X	X
Gomphonema parvulum (Kütz)	X	X	X	X
Gomphonema species of unknown or uncertain identity				X
Meridion circulare var. *circulare* (Grev.)	X	X	X	X
Navicula placentula Ehr.	X		X	X
Navicula species of unknown or uncertain identity	X			X
Nitzschia species of unknown or uncertain identity			X	X
Pinnularia gibba var. *mesogongyla* Ehr.	X	X	X	X
Planothidium lanceolatum var. *dubia*	X		X	X
Planothidium lanceolatum var. *frequentisima*	X			X
Planothidium lanceolata var. *lanceolata* (Bréb.) Grunow	X	X	X	X
Stauroneis species of unknown or uncertain identity				X
Staurosira species of unknown or uncertain identity		X	X	X
Surirella species of unknown or uncertain identity				X

lower than at Barnett and Brown Springs (9.2 and 8.5 mg L^{-1}, respectively). Percent saturation ranged from 70% in Mint Spring water to 80% in Brown Spring. Alkalinity at Mint and Panther Springs (1.65 and 2.09 mEq L^{-1}, respectively) was considerably higher than at Barnett and Brown Springs (0.15 and 0.20 mEq L^{-1}, respectively). pH at Mint and Panther Springs was higher (7.2 and 7.4, respectively) as compared with 6.4 and 6.2 at Barnett and Brown, respectively.

There were no differences among the springs in ammonium or silica concentrations (Table 3). NO_3^- + NO_2^- concentrations were much higher in Barnett and Brown springs (0.144 and 0.123 mg L^{-1}, respectively) compared with Mint and Panther springs (0.053 and 0.070 mg L^{-1}, respectively) as were SO_4^{2-} concentrations in Barnett and Brown springs (5.54 and 10.87 mg L^{-1}, respectively) compared with Mint and Panther springs (2.11 and 2.94 mg L^{-1}, respectively). PO_4^{3-} concentrations were significantly higher in Mint and Panther springs (0.092 and 0.042 mg L^{-1}, respectively) compared with Barnett and Brown springs (0.004 and 0.006 mg L^{-1}, respectively), and Cl^- concentrations were found to be significantly higher in Panther and Barnett springs (2.16 and 1.76 mg L^{-1}, respectively) compared with Mint and Brown springs (1.65 and 1.6 mg L^{-1}, respectively).

Cluster analysis showed that there were very distinct taxa compositions at each spring (Figure 3). Cluster analysis also showed that Mint and Panther springs were similar in physicochemical and nutrient variables (Figure 4). Mint and Panther springs had significantly higher concentrations of PO_4^{3-} (and significantly lower concentrations of NO_3^- + NO_2^-) compared with Barnett and Brown springs. Barnett and Brown springs differed from each other more in their physicochemical and nutrient variables. Although there was a distinct dominance of certain taxa at each spring, there were taxa present that comprised less than 1% of the total abundance (Table 1).

Principle components analysis (PCA) was performed on all 13 environmental variables. Those with eigenvalues greater than 0.7 were selected as variables that explained the most variance in species composition at each spring (Table 4). Six variables selected by the anal-

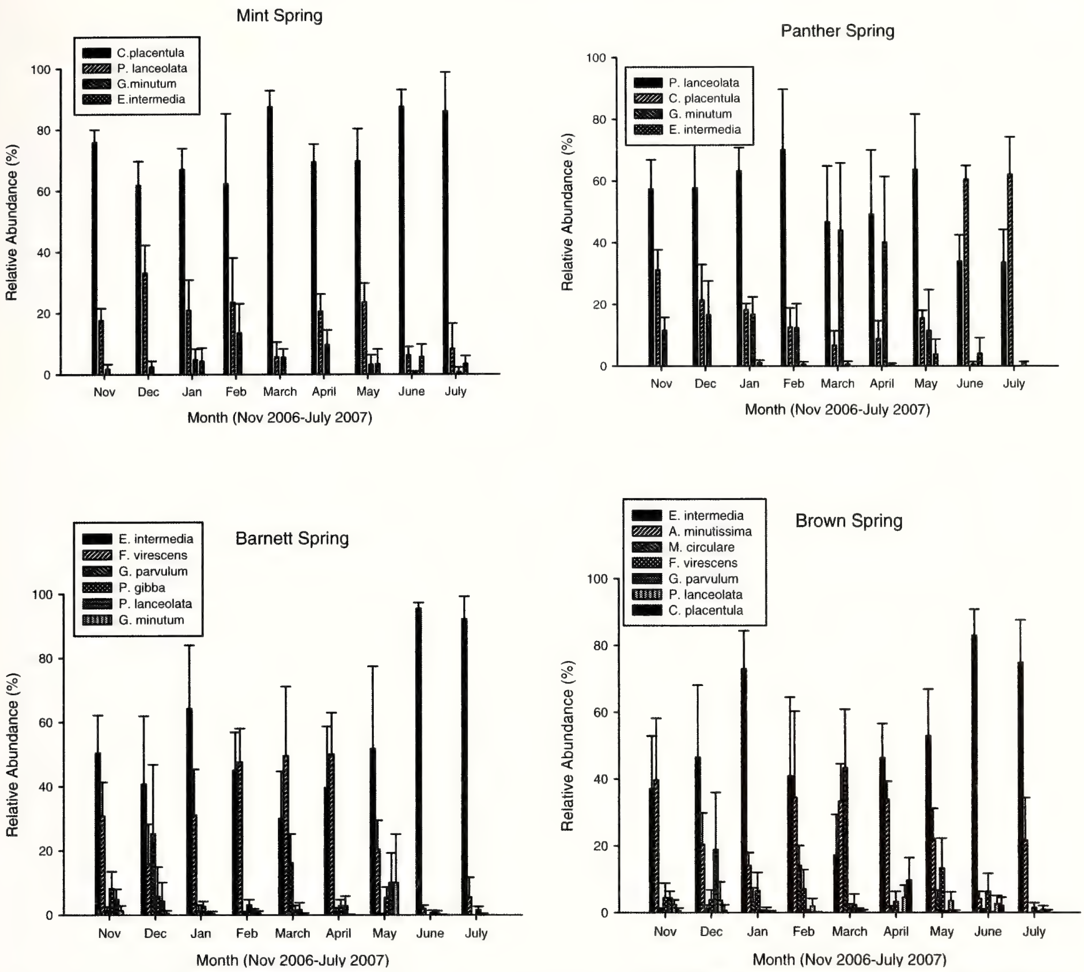

Figure 2. The relative abundance of taxa found at each spring in Land-Between-the-Lakes National Recreation Area, western Kentucky and Tennessee.

ysis were conductivity, alkalinity, PO_4^{3-}, SO_4^{2-}, NO_3^- + NO_2^-, and pH. PCA results showed the component loadings for the most important environmental variables (Table 5). Conductivity, alkalinity and PO_4^{3-} explained 68% of the variance in species composition at each spring. The first component axis explained 41.6% of the total variance in species composition between the springs. The second component axis explained 15.6% of the total variance. The component loadings showed that conductivity, alkalinity, PO_4^{3-}, SO_4^{2-},

Table 2. Comparison of mean physicochemical variables (s.d.) among four springs in western Kentucky and Tennessee (Land-Between-the-Lakes Natioanl Recreation Area). Means marked with the same letters (a–d) are not significantly different from each other ($P \geq 0.05$).

Variable	Mint	Panther	Barnett	Brown
Conductivity ($\mu S\ cm^{-1}$)	205.4^a (4.8)	250.8^b (14.8)	34.2^c (3.5)	53.5^d (7.6)
DO ($mg\ L^{-1}$)	7.1^a (0.4)	7.9^b (0.6)	9.2^c (0.8)	8.5^d (0.8)
Temperature (°C)	14.8^a (0.1)	14.2^b (0.2)	14.2^b (0.5)	13.1^c (1.2)
Light ($\mu moles\ m^{-2}\ sec^{-1}$)	501^a (408)	288^a (54)	621^a (110)	581^a (107)
Alkalinity ($mEq\ L^{-1}$)	1.65^a (0.09)	2.09^b (0.15)	0.15^c (0.03)	0.20^c (0.04)
Flow ($m^3\ sec^{-1}$)	0.018 (0.006)	0.022 (0.007)	0.001 (0.000)	0.002 (0.002)
pH	7.2^a	7.4^a	6.4^b	6.2^b

Table 3. Comparison of mean concentrations (s.d.) of nutrients in four perennial springs in western Kentucky and Tennessee (Land-Between-the-Lakes). Means marked with the same letters (a–d) are not significantly different from one another other ($P \geq 0.05$). All variables are expressed in mg L^{-1}.

Variable	Mint	Panther	Barnett	Brown
PO_4^{-3}	0.092[a] (0.007)	0.042[b] (0.003)	0.004[c] (0.003)	0.006[c] (0.003)
$NO_3^- + NO_2^-$	0.053[a] (0.002)	0.070[b] (0.002)	0.144[c] (0.027)	0.123[d]
NH_4^+	0.004[a] (0.004)	0.004[a] (0.004)	0.003[a] (0.003)	0.004[a] (0.003)
SiO_2	8.717[a] (0.168)	8.661[a] (0.206)	7.207[a] (3.972)	8.611[a] (4.035)
Cl^-	1.648[a] (0.034)	2.156[b] (0.127)	1.758[c] (0.138)	1.598[a] (0.058)
SO_4^{-2}	2.112[a] (0.408)	2.937[b] (0.308)	5.539[c] (0.650)	10.868[d] (0.710)

$NO_3^- + NO_2^-$, and pH were all highly correlated with the first axis and that pH and SO_4^{2-} were correlated with the second axis.

Using the six variables that explained the most variance in species composition, a canonical correlation analysis (CCA) was performed to determine the strength of the relationship among the most abundant species and the physicochemical variables at each spring. Based on the CCA (Figure 5), diatom species compositions at Mint and Panther Springs were most influenced by conductivity, alkalinity, pH, and PO_4^{3-} concentrations. Species composition at Barnett Spring was most influenced by $NO_3^- + NO_2^-$ concentrations, while the species composition at Brown Spring was most influenced by SO_4^{2-} concentrations.

Of the seven most abundant taxa, each had distinct correlations with one or more water quality parameters (Table 6). Spring water conductivity, alkalinity, pH, PO_4^{3-}, $NO_3^- + NO_2^-$ and SO_4^{2-} concentrations appeared to have the most influence on diatom species assemblages. *Achnanthidium minutissimum* was negatively correlated with pH and positively correlated with SO_4^{2-} concentrations. *Cocconeis placentula* was positively correlated with alkalinity and negatively correlated with $NO_3^- + NO_2^-$ and SO_4^{2-} concentrations. *Eunotia intermedia* was negatively correlated with conductivity, pH, alkalinity, and PO_4^{3-} but positively correlated with $NO_3^- + NO_2^-$

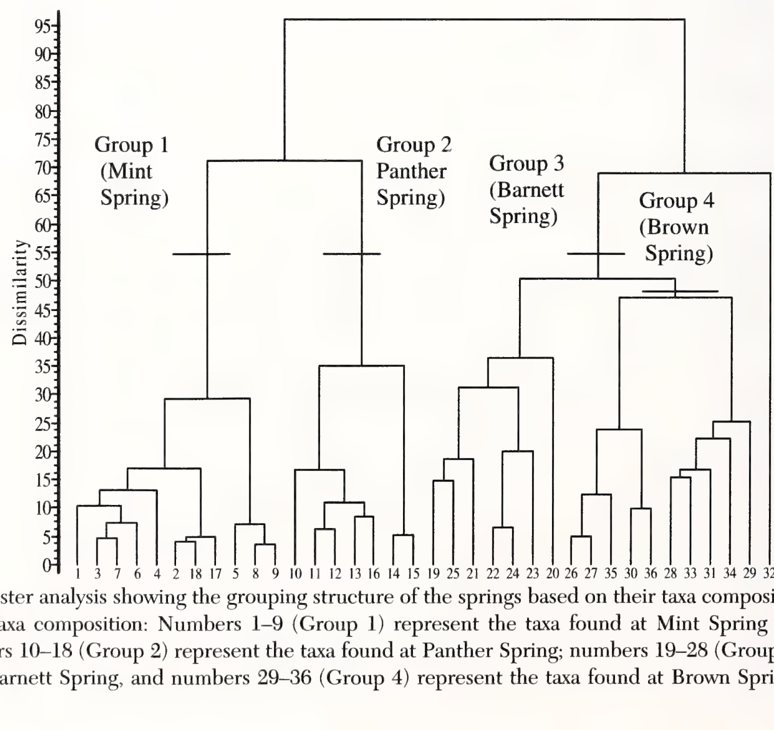

Figure 3. Cluster analysis showing the grouping structure of the springs based on their taxa composition. Each spring has a distinct taxa composition: Numbers 1–9 (Group 1) represent the taxa found at Mint Spring during the study period. Numbers 10–18 (Group 2) represent the taxa found at Panther Spring; numbers 19–28 (Group 3) represent the taxa found at Barnett Spring, and numbers 29–36 (Group 4) represent the taxa found at Brown Spring.

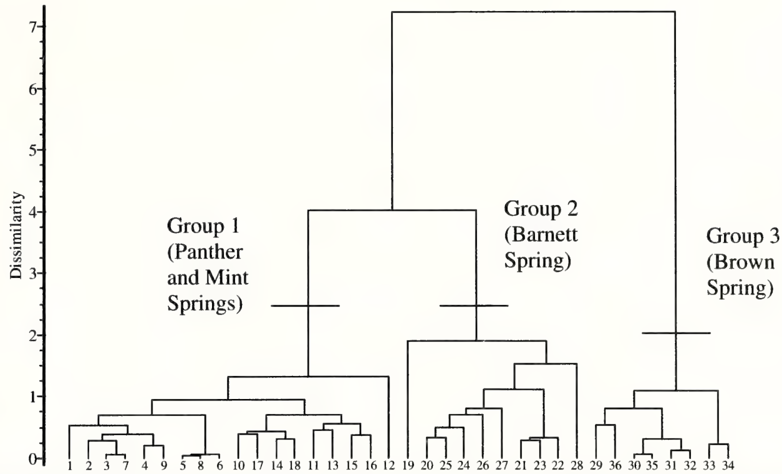

Figure 4. Cluster analysis showing the grouping structure of the springs based on their physicochemical and nutrient variables. Numbers 1–9 represent the physicochemical measurements collected for Mint Spring, November through July. Numbers 10–18 represent the physicochemical measurements collected for Panther Spring; numbers 19–28 represent the physicochemical measurements collected for Barnett Spring and numbers 29–36 represent the physicochemical measurements collected for Brown Spring. Mint and Panther spring (Group 1) are physicochemically similar; Barnett Spring is Group 2, and Brown Spring is Group 3.

and SO_4^{2-} concentrations. *Fragilariforma virescens* showed a negative correlation with conductivity and alkalinity. *Gomphonema minutum* was positively correlated with alkalinity. *Pinnularia gibba* was negatively correlated with conductivity and alkalinity, and positively correlated to NO_3^- + NO_2^-. *Planothidium lanceolata* was positively correlated with conductivity, pH, and alkalinity, and negatively correlated with SO_4^{2-} concentrations.

DISCUSSION

The diatom flora of the four spring streams was not diverse and primarily contained taxa known to be common in western Kentucky streams (Camburn 1982, Hendricks et al. 2006).

Southern LBL's Mint and Panther Springs had higher average conductivity, alkalinity, and pH indicative of their underlying limestone geology as compared to northern LBL's Barnett and Brown Springs which were underlain by siliceous and argillaceous geology. Chetelat et al. (1999) found that phosphorous concentrations were positively correlated with specific conductance, suggesting strong relationships between nutrient concentrations and drainage basin geology. Although phosphorus is usually the limiting nutrient in streams, nitrogen can be limiting in streams that have geologic sources of phosphorus, such as limestone bedrock (Hendricks and White 2000).

Barnett and Brown Springs also had much higher concentrations of SO_4^{2-} than Mint and Panther Springs. SO_4^{2-} has many sources including weathering of sedimentary rocks and atmospheric deposition (Allen 1995). Mayer et al. (2007) found that high SO_4^{2-} concentrations in stream water are caused by chemical weathering involving the oxidation of sulfides in siliceous rocks. SO_4^{2-} and bicarbonate concentrations tend to be inversely related in stream water, especially in areas where alkalinities are lower (Allen 1995).

Table 4. Physicochemical variables with eigenvalues >0.7 explaining the highest percentage of total variance in species composition among four perennial springs in western Kentucky and Tennessee (Land-Between-the-Lakes).

Component	Eigenvalue	Percentage of Variance Explained
Conductivity (ln)	6.241	41.6
Alkalinity	2.337	15.6
PO_4^{-3}	1.657	11.0
SO_4^{-2}	1.078	7.2
NO_3^- + NO_2^-	1.030	6.9
pH	0.797	5.3

Table 5. Principle Components Analysis results showing the component loadings (correlations of each variable on each component) for the environmental variables explaining the most variance in species composition among four springs in western Kentucky and Tennessee (Land-Between-the-Lakes National Recreation Area).

Variable	Component 1	Component 2
Conductivity (ln)	0.951	0.061
Alkalinity	0.950	0.111
PO_4^{3-}	0.840	0.057
SO_4^{2-}	−0.836	−0.228
$NO_3^- + NO_2^-$	−0.791	0.052
pH	0.761	0.349

Since atmospheric deposition of SO_4^{2-} has decreased over the past 20 years in Kentucky Lake (Yurista et al. 2004), it is likely that geologic weathering is the main source of SO_4^{2-} in LBL streams. Although we do not know if specific sulfide minerals such as pyrite (FeS_2) are proximate to any of the springs, LBL has been known historically for it's low-grade iron mining activities (Harris 2002) whereby such minerals may have been important.

The most abundant species in Mint and Panther Springs (*Cocconeis placentula*, *Planothidium lanceolata*, and *Gomphonema minutum*. *C. placentula* and *P. lanceolata* (syn. *Achnanthes lanceolata*)) are classified as being alkaliphilous occurring at pH 7 with best development over pH 7 (Lowe 1974). *C. placentula* has an optimal conductivity of 270 µS cm^{-1} and *P. lanceolata* has an optimal conductivity of 286 µS cm^{-1} (Potapova and Charles 2003). Many species of *Gomphonema* are classified as indifferent with best development around pH 7 (Lowe 1974). *G. minutum* has been found to have an optimal conductivity of 324 µS cm^{-1} (Potapova and Charles 2003).

Barnett and Brown springs both had abundances of *Eunotia intermedia*. Many species of *Eunotia* have been described as acidophilous (occurring at pH 7 with best development below pH 7) to indifferent (Lowe 1974), and many species have an

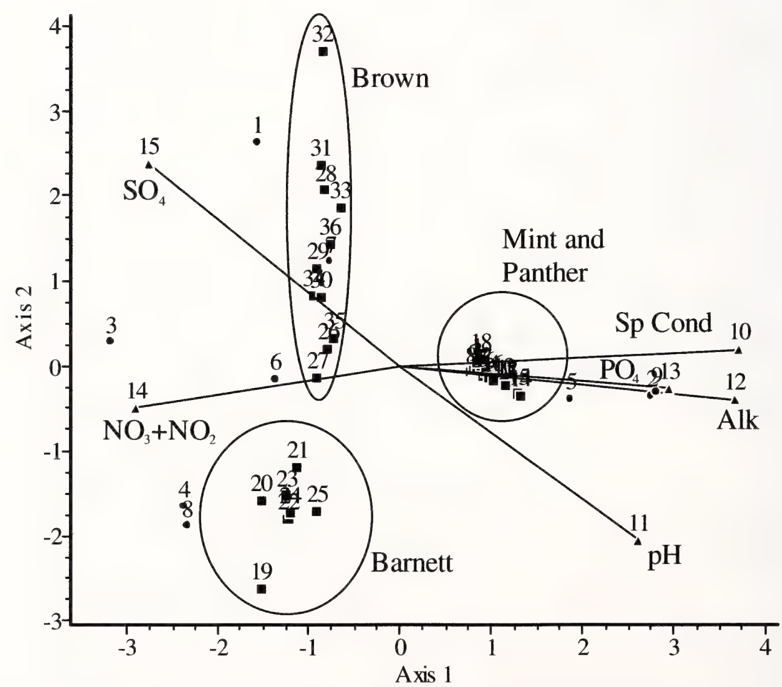

Figure 5. Triplot based on the canonical correlation analysis. The circled numbers represent the taxa found at each spring November through July. Numbers 1–18 represent the taxa found at Mint and Panther springs. Numbers 19–27 represent the species composition at Barnett Spring. Numbers 28–36 represent the taxa found at Brown Spring. Each line represents the physicochemical variables which had the most influence on the taxa composition at each spring (conductivity, alkalinity, pH, PO_4^{-3}, $NO_3^- + NO_2^-$, and SO_4^{-2}).

Table 6. Canonical correlation results showing the correlation coefficients between the most abundant species and the physicochemical and nutrient variables at each spring. Genus and species names are found in Table 1. (*) represents correlation coefficients ≥0.5.

Variable	*A .min*	*C. lin*	*E. int*	*F. vir*	*G .min*	*G. par*	*M. cir*	*P. gib*	*P. lan*
Conductivity	−0.376	0.730*	−0.857*	−0.647*	0.487	−0.361	−0.179	−0.647*	0.752*
pH	−0.683*	0.473	−0.631*	−0.144	0.449	−0.377	−0.255	−0.154	0.664*
Alkalinity	−0.482	0.720*	−0.856*	−0.565*	0.528*	−0.350	−0.226	−0.565*	0.765*
PO_4	−0.421	0.927*	−0.721*	−0.490	0.210	−0.298	−0.188	−0.479	0.307
$NO_3 + NO_2$	0.215	−0.730*	0.833*	0.443	−0.279	0.199	−0.044	0.551*	−0.465
SO_4	0.766*	−0.672*	0.682*	0.215	−0.393	0.202	0.400	0.171	−0.554*

optimal conductivity range from 48 to 116 µS cm^{-1} (Potapova and Charles 2003).

Barnett Spring had a good population of *Fragilariforma virescens* (syn. *Fragilaria virescens*). *F. virescens* is classified as indifferent (Lowe 1974) and species of *Fragilarioforma* have an optimal conductivity of 86 µS cm^{-1} (Potapova and Charles 2003). *Achnanthidium minutissimum* (syn. *Achnanthes minutissima*) which has been classified as indifferent (Lowe 1974) with an optimal conductivity of 229 µS cm^{-1} (Potapova and Charles 2003) was common in Brown Spring. Because the pH and conductivity of Brown Spring do not meet the optimal environmental conditions for *A. minutissium*, it is possible that the high SO_4^{2-} concentrations had a positive influence on the abundance of this species at this spring. Bonny and Jones (2007) found that *A. minutissimum* was abundant in springs with high SO_4^{2-} concentrations. It is also important to note that the abundance of *Meridion circulare* was very high in the month of March in Brown spring. Although *M. circulare* generally achieves maximum abundance during the spring (Krecji and Lowe 1987) and is considered an alkaliphilous species (Lowe 1974), it remains unclear why it became so dominant.

There were many taxa present at each spring that never became dominant. All of these taxa occur at a pH 7, but the conditions might not have been optimal in other ways for their development. For example, many species of *Amphora* are classified as alkaliphilous (Lowe 1974), but the optimal conductivity for each are very high, ranging from 515–634 µS cm^{-1} (Potapova and Charles 2003). The extremely high optimal conductivity requirements for *Amphora perpusilla* could be one reason why it did not become a dominant species in Mint Spring. Many species of *Caloneis* are also classified as alkaliphilous (Lowe 1974) and have high optimal conductivities (>365 µS cm^{-1}, Potapova and Charles 2003). We conclude that these environmental conditions were not met in Barnett Spring for *Caloneis hyalina*. Optimal environmental conditions for many species of *Cymbella* vary greatly. They have been classified as acidophilous, indifferent, alkaliphilous and alkalibiontic (occurring only in alkaline water) (Lowe 1974). Their optimal conductivity range is 89–347 µS cm^{-1} (Potapova and Charles 2003). *Cymbella* was present in both Brown and Barnett Springs but was never abundant probably because of non-optimal conductivities.

In summary, it is apparent that most of the dominant species occurring in the southern LBL springs also were present (but not dominant) in the northern LBL springs. Likewise, most of the dominant species in the northern springs were present (but not dominant) in the southern springs.

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Assessing the Impacts of Coal Waste on Residential Wells in the Appalachian Region of the Big Sandy Watershed, Kentucky and West Virginia: An Exploratory Investigation

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ABSTRACT

This paper examined issues surrounding coal waste and its potential impacts on residential private wells by reviewing the existing literature to identify the possible issues and parameters associated with coal waste and its possible effects on private wells. Using well water data from the Big Sandy Region of Kentucky and West Virginia (n=42), drinking water quality was examined using standard heavy metal parameters associated with coal waste: arsenic, cadmium, chromium, iron, lead, manganese, zinc, and sulfate. Findings showed significantly more wells in sub-watersheds with coal waste impoundments with iron levels above secondary drinking water standards. A review of similar wells from the Kentucky Groundwater Database Repository showed a similar trend. This pattern warranted further study of Fe as a possible coal slurry waste marker. Other general findings revealed high concentrations of manganese, lead, and arsenic across our sampling of cases. Levels of these metals were high in Appalachian rock, so linking their levels to coal mining is problematic. Overall, findings suggested that residential well water in the coal mining area of the Big Sandy region of Appalachia may be of variable and sometimes unhealthy quality.

KEY WORDS: Coal waste, impoundments, well water, drinking water quality

INTRODUCTION

The Appalachian region of eastern North America is a major coal producing region (Figure 1; US DOE 2006). On numerous occasions, residents of this region have voiced concern for the effects of coal mining on various aspects of their lives, especially the ground water used by many residents (McSpirit et al. 2005, 2007; Scott et al. 2005). Mining activities that have generated the most concern include mountain top removal, coal slurry impoundments, and the injection of coal wastes into deep mines (Stout and Papillo 2004; WV Joint Judiciary Subcommittee 2006).

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Coal wastes can contain a variety of metals that can cause health effects, including arsenic, barium, cadmium, chromium, lead, selenium, silver and copper, iron and manganese. The US EPA concluded that the coal wastes injected into deep mines could be a source of metal contamination if they entered an aquifer (US EPA 1985). Poorly managed coal waste impoundments may generate acid which can increase the solubility of heavy metals in coal waste (Brady 1998). In West Virginia, concerns over deep-mine disposal methods have lead to legislative hearings and a directive to study these concerns more deeply (WV Joint Judiciary Subcommittee 2006; HNN Staff 2007).

In October, 2000 a 68-acre reservoir released impounded slurry and coal waste

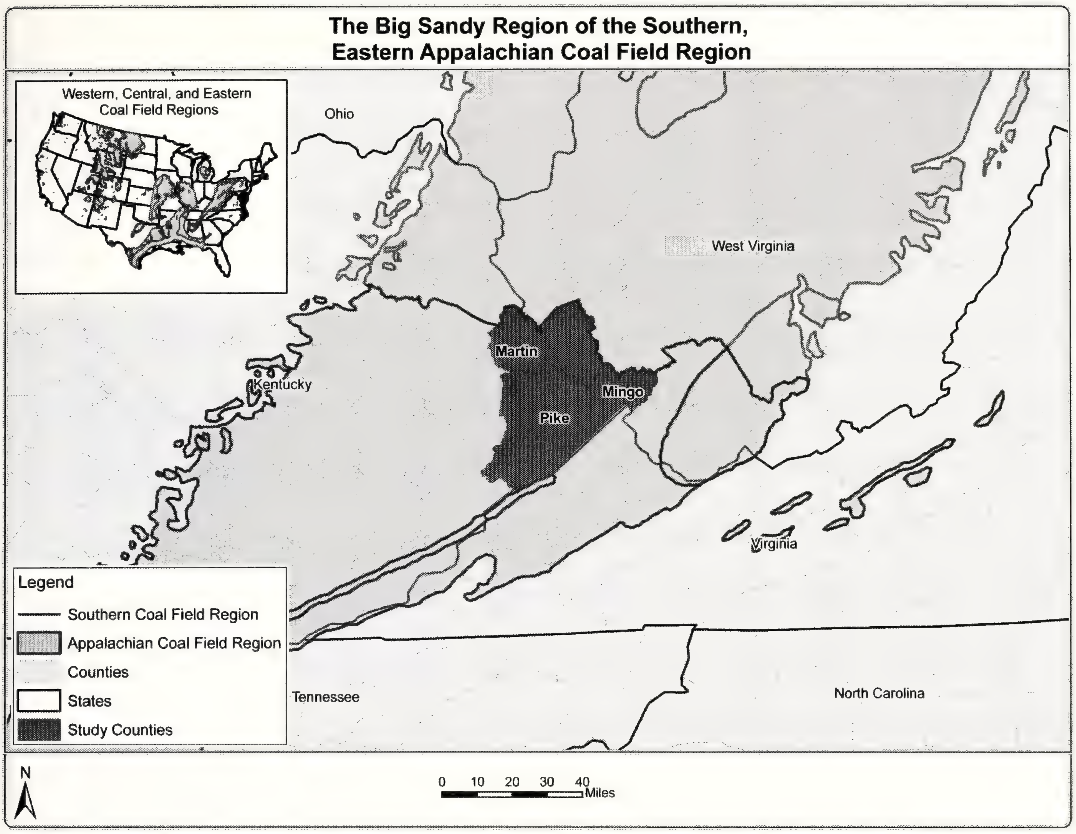

Figure 1. The Big Sandy Region of the Southern, Eastern Appalachian Coal Field Region.

into underground mines that then blew-out two mine portals, releasing an estimated 300 million gallons of slurry and sludge waste into area creeks and water ways in Martin County, KY (US DOI 2002). Environmental settlement money from the spill was used for assessments of the local reservoir, the local water company, and metal levels in resident's homes. Several wells were examined in the latter study that led to our greater interest in the possible effects of the Martin County spill and other coal mining activities on well water quality.

The US EPA (1985) and USGS (1989) in past site assessments have identified the following constituents as associated with coal slurry waste: arsenic, cadmium, chromium, iron, lead, zinc, manganese and sulfate. In general, we were interested in exploring whether some of these chemicals could be used in monitoring and tracing possible coal waste impacts and differences between sites in coal impacted areas. Therefore, it might be expected that more downstream wells in sub-watersheds with coal waste impoundments would experience lower drinking water quality than other wells in our study area in Southern Appalachia.

METHODS

Considering the possible impacts of coal waste on the environment and human health, our principle research question was to explore possible coal waste effects on drinking water from residential wells. While our previous findings reinforced past US EPA and ATSDR assessments that indicated no long term effects of the 2000 event on the county public water system (Lasage and Caddell 2006; McSpirit and Wigginton 2006; Wigginton et al. 2007), the next question was whether private, residential wells had been affected. Although early sampling and testing by state regulatory agencies had shown tested wells to be within acceptable drinking water standards (KY DOW 2000), many residents in the

impacted area of Martin County have remained concerned about the possible slow migration of heavy metals and other constituents into their aquifers from coal waste and coal waste impoundments.

Each of our three study sites was within the Big Sandy watershed region of Kentucky and West Virginia (Figure 1) in an area with coal waste impoundments and other coal mining activities such as surface mining or deep mine slurry injection. The Big Sandy region starts the area of southern Appalachia, which, according to USGS reports, is less prone to acid mine drainage (AMD) due to more calcareous and carbonate-based strata than northern Appalachia (USGS 1989). However, the Big Sandy region has been the site of the three previously cited coal waste cases that have been handled by the US EPA or ATSDR. Accordingly, we focused our analysis on three sites within this region.

Site 1, Martin County, Kentucky, was the site of the 2000 coal waste release, where the bottom of the Big Branch Slurry impoundment breached into underground mine works, releasing over 300 million gallons of coal slurry and sludge material into area waters. Martin County has a small land area and population of only 12,093 people (U.S. Census Bureau, State and County Quick Facts, http://www.census.gov/). The principal economic activity is coal mining. In 2005, there were 11 active mining operations with 3 surface permits and 8 underground mining operations in 2005. Together, they produced 5.3 million - tons of coal that year (U.S. Department of Energy 2006). Besides the Big Branch Slurry impoundment, there are 4 other coal waste impoundments in the county based on online data from the Coal Impoundment Project (Coal Impoundment, Location and Information System (LIS) http://www.coalimpoundment.org/).

Whereas other studies had used more focused approaches, we applied more random methods in data collection to avoid any bias in our resulting data. During late summer (August and September), well water samples were collected based on door to door solicitations by our field from 24 wells across the county (Figure 2). Given the length of time since the 2000 coal slurry release, it is possible that this event or other smaller incidences of "black water" have impacted the aquifers throughout the county and hence, the rationale for our focus on a wide geographic scope of the county.

Site 2, Mingo County, West Virginia, also is heavily involved in coal production and lies parallel to Martin County, on the other side of the Tug Fork of the Big Sandy River. In 2005, there were 28 active mining operations, 15 deep mines and 13 active surface or mountain-top removal sites that produced 14.5 million tons of coal (U.S. Department of Energy 2006), as well as 12 coal waste impoundments (Coal Impoundment, LIS). The population of Mingo is higher than that of Martin County with 27,100 residents (US Census Bureau). The Rawls, Merrimac and Lick Creek area (near Williamson, WV) was the site of the most recent ATSDR investigation into the effects of deep mine slurry injection methods on domestic wells which resulted in a warning for infants and those with liver and gastrointestinal disease to refrain from drinking from domestic supplies due to high concentrations of manganese (ATSDR 2004, 2005).

In this case, we refrained from sampling in the Rawls, WV, area due to a pending civil suit between residents and the coal company. For a discussion of data and findings from this area, see ATSDR (2004); Stout and Papillo (2004), and ATSDR (2005, -a response to Stout). In collecting data on other places in the county, we relied on a field guide who was a member of a concerned citizen's group. This more focused method allowed us to collect from 11 wells in mid- December 2006 (Figure 2).

Site 3 in Pike County, Kentucky, has a population of 66,860 people (U.S. Census). It is the principal coal-producing county in Kentucky. In 2005, there were 112 active mine operations in Pike County (68 underground, 44 surface mines) and they produced 28.4 million tons of coal (U.S. Department of Energy 2006). There are 17 coal waste impoundments in this county (Coal Impoundment, LIS). It also was the county where the 1985 coal waste injection case took place in which the US EPA concluding that an "imminent and substantial" threat existed to groundwater as a consequence of these deep-mine disposal methods.

Our specific study site was a far distance from that site. In Pike County, we collected well water samples from homes that were

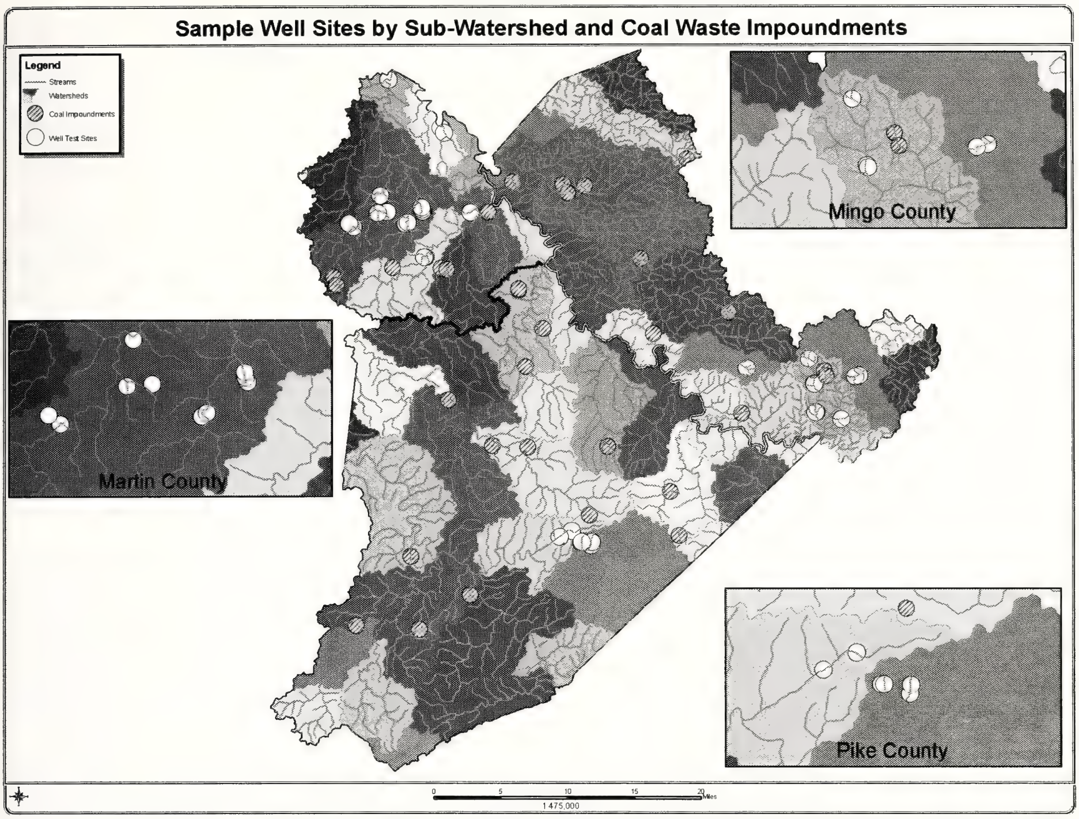

Figure 2. Sample Well Sites by Sub-Watershed and Coal Waste Impoundments.

directly down gradient from an active mountain-top removal site. Residents in this area have complained about damage and contamination from these operations to their private wells, but these complaints do not revolve specifically around coal waste. Well water samples, in this case, were collected based on communication with a concerned resident who then served as our field guide. With their assistance, we collected samples from 8 wells in and around a small hamlet in mid October 2006. Water samples were collected from a total of 42 domestic wells from Martin, Mingo, and Pike counties during low-flow hydrological conditions of mid to late summer and mid to late fall (early winter). Five samples were to be collected on each home: a well sample for detailed analysis, a second well sample for portable test kit analysis, cold water tap, hot water tap, and water heater drain valve. Some homes could not be sampled at all locations because of poor access, especially for water heaters. In this research, only well water data are reported in detail. Wells were sampled by choosing the outside tap closest to the well pump. Care was taken to ensure that the tap exited the line before any water softener. Water was allowed to run for several minutes until a change in temperature was noticed, indicating that any pressure tanks had been flushed and new water was being drawn from within the well casing (Haman and Bottcher 1986). In the case of one well with no pump apparatus, it was sampled with a bottle attached to a weighted line. New polyethylene (PE) bottles were used to collect all samples. After collection, sample bottles were placed in plastic, resealable bags and kept on ice until they were acidified using trace metal grade nitric acid (HNO_3; US EPA 2005).

Date and time that samples were collected was recorded in our field logs as well as other descriptive data. Global Positioning System (GPS) coordinates were logged on general vicinity (generally within 30 m) rather than exact well location. A more rigorous study of specific hydrological conditions would imply collecting GPS data on the exact location of

each domestic well and the mapping of other land and hydrological characteristics of interest on site. However, such mapping would necessitate formalizing additional consent and confidentiality agreements between researchers and university institutional review boards before such information could be recorded and catalogued on homeowners. Gathering more specific participant information that could potentially link participants to particular wells would have likely reduced the number of residents willing to work with us and hence, our rationale for collecting GPS data within the general (30 m) vicinity.

Field data were also collected on well-depth and whether wells were drilled or hand-dug. In this exploratory study, well-depth was collected based on homeowner estimates. Past USGS studies have used either driller logs and/or field probes to better measure well depth, but for our purposes residential self-reports were adequate because we were pursuing citizen based science and the cost and potential for damaging residential wells if more accurate measurement were made would have discouraged participation. We also collected background data about household size (number of people present in the household) to serve as a proxy assessment of heavy versus light water use. Information was also collected on the age of the home and plumbing type: copper, iron, galvanized steel, or polyvinyl chloride (PVC).

Analytical Parameters and Laboratory Methods

In its investigative report after the 2000 coal sludge event, the National Research Council stated, as one of its recommendations, the need to characterize the constituents of coal slurry to better "aid in monitoring schemes" (NRC 2002, p. 127). In our efforts to start to characterize the constituents of coal slurry, metals were initially selected for analysis based on their prevalence in early US EPA and ATSDR reports and known presence in coal. They included arsenic, barium, cadmium, chromium, cobalt, copper, iron, lead, manganese and selenium (U.S. EPA 1985; Booth et al. 1999; ATSDR 2001; Huggins 2002; Wagner and Hlatshwayo 2005). Sulfate was also selected for analysis because it can be found in acid mine drainage or water pumped from flooded mines (Grey 1998; Batty et al. 2005; Tiwary 2001).

All samples were analyzed for metals at the Ecotoxicology and Environmental Assessment Laboratory (EEAL) at the University of Kentucky using a Varian Vista MPX Simultaneous Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) and methods of the APHA (1995). Calculated metal concentrations were corrected back to check standards. Samples collected during the field sweeps were analyzed for sulfate during May, 2007 at the Environmental Research and Training Laboratory (ERTL), also at the University of Kentucky using a Dionex ICS-2500 Ion Chromatograph and methods developed by the U.S. EPA (Method 300.0; 1993).

Geographic Information System Analysis

Prior to statistical analysis, additional geocoding methods were used in a Geographic Information System (GIS) to code wells based on sub-watershed and whether the well was in a sub watershed with an active or reclaimed impoundment. Other GIS data were also compiled based on well proximity to a deep mine site or surface mine site, but preliminary analysis showed these other factors to be statistically insignificant in explaining well water quality perhaps due, in part, to the variability in data mapping of active deep mines and surface mine sites. Also, slurry injection point data for Mingo County was viewed in a GIS, but the limited number of cases (n = 11) prevented systematic analysis on this dimension. In the conclusion of this paper, future research needs are mentioned with emphasis on developing GIS methods. For the following analysis, the main spatial tool in explaining water quality was whether a well was in a sub-watershed with either an active or reclaimed impoundment and down stream of it (Figure 2).

Statistical Methods

Although somewhat different methods were used to determine which sites to sample, it was not anticipated that this would hinder data analysis since guides could not know whether given sites were contaminated or not. Effectively, the more focused approach used in Pike and Mingo Counties simply allowed us

Table 1. General overview of findings on concentrations for heavy metals by site: findings for those metals that produced notable results reported: arsenic, iron, lead, and manganese (ppb) Non-detects set to half detection limit.

	N	Min	Q1	Mdn	Q3	Max	Mean	Std Dev	Std Err	US EPA	# Ex
Arsenic (As)											
Site 1[a]	24	2.50	2.50	2.50	2.50	14.5	3.42	2.81	0.57	10	1
Site 2[b]	11	2.50	2.50	2.50	5.33	7.19	3.50	1.76	0.53	10	0
Site 3[c]	8	2.50	2.50	4.25	8.97	1340	171	470	166	10	1
Site 3£	7	2.50	2.50	2.50	7.10	9.60	4.67	2.91	1.09	10	0
Iron (Fe)											
Site 1	24	25	257	1210	4220	17,600	3540	5100	121	300	17
Site 2	11	25	53.9	4120	12,400	31,100	6590	9350	2820	300	8
Site 3	8	25	25	25	209	3000	367	1040	367	300	1
Lead (Pb)											
Site 1	24	0.50	0.50	2.61	22.2	527	35.3	107	21.9	15	6
Site 2	11	0.5	1.15	1.55	9.59	29	5.83	8.87	2.67	15	1
Site 3	8	0.50	0.50	1.20	29.4	347	51.2	120	42.5	15	3
Manganese, (Mn)											
Site 1	24	0.25	22	70	259	870	192	255	52	50	15
Site 2	11	0.25	0.90	234	826	1300	366	234	133	50	5
Site 3	8	1.20	44	220	1290	30,500	4120	10,700	3770	50	7
Sulfate ($SO_4{}^{2-}$)											
Site 1[a]	13	1.13	2.0	4.68	35.6	147	29.2	50.9	14.1	250	0
Site 2[b]	9	2.06	10.0	28.6	86.6	132	46.6	46.3	15.4	250	0
Site 3[c]	7	8.18	14.0	37.0	81.4	93.7	47.2	36.7	13.8	250	0

[a] Site 1: Martin County, KY.
[b] Site 2: Mingo County, WV.
[c] Site 3: Pike County, KY.
[£] Site 3: Arsenic concentrations for Pike County with extreme case, unused well (1335.4 ppb) deleted from analysis.

to find homes with wells more quickly than in Martin County. For further analysis data for all three counties was pooled.

To explore our hypotheses, well data for each of the above heavy metals was also coded based on being above or below safe and secondary drinking water standards. These data were then compared for downstream wells located in an impoundment sub-watershed versus other wells in non impoundment sub-watersheds. Standard nonparametric cross tab and Chi Square tests of statistical significance were used to determine differences in the proportion of wells below or above safe or secondary drinking water standards based on sub-watershed comparisons. To confirm these findings, well data from the Kentucky Groundwater Database Repository (KGDR) were analyzed in a similar way. Wells from the same counties and from approximately the same time frame (2003–2007) were selected for analysis. To avoid artificial bias based on sampling frequency, if multiple samples were taken on a given day, one sample was selected at random and any others discarded. Unfortunately, finer level comparison of means tests and regression analyses could not be performed on the data due to the extreme variability in well water findings and due to the relatively small case base.

RESULTS AND DISCUSSION

Arsenic (As). Two wells exceeded the 10 ppb US EPA drinking water guideline for arsenic (Table 1), one each in site 1 (max=14.5 ppb) and site 3 (max=1340 ppb). The extreme value for site 3 was a sample drawn from a well that is no longer in use (other wells on this property were within drinking water standards for As.) With this unused well dropped from the analysis, findings show one other well at site 1 (14.5 ppb) with As concentrations above US EPA 10 ppb safe standards. Samples taken from cold water taps were also analyzed and while results for most residences were within acceptable ranges (=1.85 ppb ± SD =3.07), one location (Mingo County) had cold tap water with levels above safe drinking water

standards for arsenic (11.4 ppb). This may suggest the need for further study of well water for arsenic exposures within this particular area, especially in light of extreme As results from site 3, which are discussed in more detail below. High levels of As in groundwater are common in Appalachia. Shiber (2005) found that 6% of tap water samples from residences with private wells had As levels in excess of 10 ppb.

To confirm the extreme findings from the location in site 3, the well was sampled a second time six months later. Arsenic levels from this unused source still exceeded the guideline value, but at 12.2 ppb. During the second site visit, it was noted that the water level in the 55 m deep well had changed dramatically from nearly dry during the first sampling (October) to having a water table 21 m deep (May). Based on independent well water tests conducted during the 1980s provided by the home owner, water levels were relatively stable at approximately 8 m deep. Though arsenic concentrations are not recorded on these logs, since the 1980s, this area of Pike County has experienced heavy surface (Mountaintop removal) and continued deep mining which has likely altered the hydrology of the area greatly over the past 20 years. Moreover, Kentucky coals typically exceed mean arsenic concentrations for U.S. coals and this area of Pike County has recorded high As concentrations in stream sediment (58–110 ppm) (Tuttle et al. 2002). While other wells at site 3 were within safe drinking water standards, the reported exceedances and human health effects associated with As exposure (Smith 1992, 1998; Gomez-Caminero et al. 2001; Kinniburgh and Kosmus 2002) suggests that further study may be warranted.

Iron (Fe). Median iron values for site 1 (1210 ppb) and site 2 (4120 ppb) far exceeded US EPA secondary standards (300 ppb) (Table 1). In fact, 17 of 24 wells (71%) for site 1 and 8 of 11 wells (73%) for site 2 exceeded secondary Fe standards (300 ppb). In comparison, only 1 of 8 wells was in exceedances (max=3000 ppb) at site 3. Maximum exceedances at site 1 (17,600 ppb) and site 2 (31,000 ppb) ranged from 58 to over 100 times secondary drinking water standards. While all sites (1,2 and 3) are known for above average iron concentrations in soils and sediments (Gustavsson et al. 2001), high Fe might not only be associated with area soils, but also pyrite (FeS_2), a natural component of coal, which may also contribute to water bourn levels of Fe. Water from coal seams is sometimes high in Fe (Wunsch 1992). Moreover, magnetite (Fe_3O_4), a major component of the coal cleaning process and coal waste impoundments, may also be a source contributor. In the next set of analyses, the possible effects of coal waste on well water and iron levels is modeled through GIS analysis of sub watersheds.

Lead (Pb). Mean concentrations for lead are higher in KY counties (Table 1, site 1 and 3) and were at least twice the level US EPA safe drinking water standards (US EPA = 15 ppb; site 1 $\bar{x}$ = 35 ppb; site 3 $\bar{x}$ = 51 ppb). Maximum exceedances across both sites (site 1 = 527 ppb and site 3 = 347 ppb) were well over 20 times safe drinking water standards set for lead. Due to these extraordinarily high exceedances, and the well-substantiated health effects of lead exposure on human health, cold water tap results were also examined. Analyses showed coldwater samples to be within more or less acceptable ranges ($\bar{x}$ = 4.23, SD = 14.7). However, two coldwater (drinking water) samples reported lead levels above safe standards (25.3 ppb; 92.6 ppb respectively).

Manganese (Mn). As with iron, elevated levels of Mn can be associated with coal seams and mine drainage, possibly because of sulfide mineral oxidation (O'Steen and Rauch 1983; Wunsch 1992). Some parts of Appalachia have Mn soil concentrations well above the national average (Gustavsson et al. 2001). Across all three sites (Table 1), more than 60% of tested domestic wells exceeded US EPA secondary standards set for manganese (50 ppb). Median concentrations were in exceedance and were nearly 8× secondary standards across two of the three sites (site 1, mdn = 70, site 2, mdn = 234; site 3, mdn = 220). Median values and exponentially higher mean concentrations (site 1 $\bar{x}$ = 870 ppb; site 2 $\bar{x}$ = 1300; site 3 $\bar{x}$ = 30,500) indicate manganese drinking water exposures of possible health concern as addressed by ATSDR in its 2005 site assessment of Mingo County (ATSDR 2004). In recent West Virginia hearings, health officials

Table 2. Comparison of several heavy metal parameters below or above drinking water standards for reference wells in sub-watersheds with no coal waste impoundments (reference wells) versus wells in sub-watersheds with one or more coal waste impoundments (n = 42).[a]

	SDWS / DWS[b]	Reference	Impoundment Sub-watershed	
Iron (Fe)				
Below SDSW(%)[b]	300 ppb	15 (54)	2 (14)	X^2 = 5.98, *df*= 1, *sig* = .015*
Above SDWS(%)		13 (46)	12 (86)	
Lead (Pb)				
Below DWS (%)	15 ppb	21 (75)	11 (79)	X^2 = .066, *f* = 1, *sig* = .798
Above DWS (%)		7 (25)	3 (21)	
Manganese (Mn)				
Below SDWS (%)	50 ppb	11 (39)	5 (36)	X^2 = .05, *df* = 1, *sig* = .822
Above SDWS (%)		17 (61)	9 (64)	

[a] Site 1: Martin County, KY - Site 2: Mingo County, WV - Site 3: Pike County, KY.
[b] SDWS = US EPA secondary drinking water standard; DWS = US EPA safe drinking water standard.
* $p \leq .05$.

have raised similar concerns regarding high Mn concentrations in exceedance of secondary standards (WV Joint Judiciary Subcommittee Hearing 2006, testimony of toxicologist, Dawn Seeburger). Manganese findings across all three active coal mining sites, not only site 2 (Mingo County), indicate Mn concentrations to be far in exceedance of secondary standards and therefore, coldwater (drinking water) samples were also checked for Mn levels. While median levels were within acceptable levels (11 ppb) for all three sites, the overall mean concentration was high suggesting wide variability in cold water results ($\bar{x}$ = 136 ppb ± SD = 261 ppb). Thirty-six percent of cold water, drinking water samples were above 50 ppb guidelines; 5 cold tap samples were 10 to 20 times in exceedance of secondary standards (537 to 1245 ppb). Unfortunately, little work has been done on the possible health effects of such high levels of manganese in drinking water in Appalachia, although the ATSDR did recommend that certain groups avoid drinking groundwater with high levels of Mn (ATSDR 2004, 2005).

Sulfate (SO_4^{2-}, Table 1). Wells across all three sites had average sulfate concentrations below US EPA drinking water secondary standards (250 ppb). Moreover, maximum values were also well below US EPA standards at each site (site 1, max = 147 ppb; site 2, max = 132 ppb; site 3, max = 94 ppb).

It was predicted that wells in sub-watersheds with impoundments (Table 2) would have a higher proportion of wells above US EPA safe (DWS) or secondary (SDWS) drinking water standards than other reference wells that are outside impoundment sub-watersheds (see Figure 2). Yet, due to the nature of the statistical tests (cross tab and Chi Square tests), analyses could not be conducted on arsenic, or sulfate due to an insufficient number of cases above safe or secondary standards. Therefore, iron, lead and manganese are used as possible parameters, with concentrations collapsed into those above and below US EPA primary or secondary drinking water standards to compare across impoundment and non-impoundment sub-watersheds. Finally, only those residential wells expressly used for drinking water were used in this analysis (n = 42).

Findings are first reported for iron (Table 2). It was expected that iron might be more prevalent in wells in sub-watersheds with coal waste impoundments due to iron being a possible by-product of coal waste because various iron compounds are present in coal naturally (e.g., pyrite, FeS_2) and due to the coal preparation process (Magnetite, Fe_3O_4). Findings (Table 2) suggest some support for this proposition insofar as a higher proportion of wells (86%) in coal waste impoundment sub watersheds had iron concentrations exceeding US EPA secondary standards (300 ppb). In comparison, a lower proportion (46%) of wells outside impoundment sub-watersheds reported iron concentrations above secondary standards. This difference was statistically significant (X^2 = 5.98, df = 1, sig = 0.015) suggesting that wells near coal waste impoundments are more likely to experience poor drinking water quality because of iron exceedances. This conclusion is supported by the fact that well data from

the Kentucky Groundwater Database Repository show a similar pattern with 84% of wells in sub-watersheds with impoundments having iron levels above the US EPA secondary standard. In sub-watersheds with no impoundments, 66% of wells exceeded the same limits.

Findings are next reported for lead and manganese (Table 2): Here too it was predicted that lead and manganese might be more prevalent in wells in sub-watersheds with coal waste impoundments due to both being a notable parameter of coal waste in past regulatory assessments. However, on these two parameters, findings suggest the need for further exploration and study. Sub-watershed comparisons do not reveal either parameter to be a significant predictor of possible coal waste effects. For lead, a similar proportion of wells in impoundment sub-watersheds (21%) in comparison to other wells (25%) reported levels above safe drinking water standards and thus, there was no statistically significant difference between wells near impoundments and other wells with respect to lead levels above safe drinking water standards (X^2 = 0.066, df = 1, sig = 0.798). Similar non significant findings (X^2 = 0.05, df = 1, sig = 0.822) are reported for manganese. Findings show a similarly high proportion of wells in both impoundment (64%) and non impoundment (61%) sub-watersheds reporting manganese levels above secondary drinking water standards. Yet, interestingly, when well data from the Kentucky Groundwater Database Repository were compared for manganese, 90% of wells from sub-watersheds with coal waste impoundments were above the US EPA secondary drinking water limit while 69% of sub-watersheds lacking impoundments had high manganese levels.

The rock found in conjunction with coal, often called over burden, if it must be removed in the course of coal mining, may be a source of heavy metal in addition to the coal itself. The shale and sandstone of Appalachia have higher concentrations of Cd, Cu, Co, Cr Fe, K, Mn, Mo, Ni, Pb, S, Ti, and Zn than the national average for these rock types (Bogner and Sobek 1979). The occurrence of various metal sulfides is very common in Appalachian rock. The greater the availability of oxygen to these sulfides in a wet environment, the greater their solubility often is (Morgan et al. 1992). Settling pond materials are especially susceptible to dissolution because they are fine grained and homogeneous (Bogner and Sobek 1979). Additionally, metal sulfide particles can accumulate in the lighter waste portion of the coal cleaning process (Finkelman 1979). Given that much rock in the Appalachian region is higher than average in various heavy metals, it is possible that the higher levels of Mn and Pb found in the present study were not related to coal mining and simply reflect the influence of the rock through which the water passed. However, coal mining creates conditions that optimize for the mobilization of metals, either from coal, coal waste, or overburden, by crushing these materials into smaller particles, thus increasing surface area and access to oxygen. Bienkowski indicated that wells with their bottoms above the level of the local stream had lower pH and alkalinity, and higher concentrations of Mn, Fe, sulfate, and total dissolved solids, due largely to increased oxygen availability (1990). The same author indicated that the source for the Fe and sulfate in such cases was pyrite from coal (Bienkowski 1990). Especially interesting is the trend of increased Mn levels in wells from the Kentucky Groundwater Database Repository in watersheds with coal waste ponds. Manganese is often highly mobile and can correlate with Fe levels in ground water, so it too may be useful as an indicator of mining activity (Bogner and Sobek 1979; Bienkowski 1990). These factors indicate that while this study cannot offer conclusive evidence about the role of coal mining in the high levels of some metals found in Appalachian water, mining activities should be considered an important candidate for their source. A more rigorous study covering a larger number of wells would be needed to confirm these possibilities as well as offer insight into less commonly encountered metals such as Pb.

SUMMARY

In its report after the 2000 coal sludge event, the National Research Council stated, as one of its recommendations, the need to characterize the constituents of coal slurry to better assist in monitoring and regulating coal

waste and coal waste impoundments (2002). In our efforts to start to characterize coal slurry constituents, metals were initially selected for analysis based on their prevalence in early US EPA and USGS coal slurry reports (US EPA 1985; USGS 1989). Our original research question was to identify possible markers associated with coal waste by comparing well water in known coal waste impoundment sub-watersheds with other wells in our study area of the Big Sandy Watershed. Our findings did show significantly more wells in impoundment sub-watersheds with iron levels above secondary drinking water standards. This significant pattern may warrant further study of iron as a possible marker of coal slurry waste because various iron compounds are present in coal naturally (e.g., pyrite, FeS_2) and magnetite (Fe_3O_4) is a major component of the coal cleaning and preparation process and a known waste byproduct of coal slurry. Well data from the Kentucky Groundwater Database Repository (KGDR) showed the same overall pattern and also showed a similar pattern for manganese that our data did not. Further work with manganese may be justified as well because of conflicting findings.

Yet, irrespective of significant differences between sub-watersheds, overall high concentrations of iron, as well as manganese and lead and sometimes arsenic suggests that residential well water in the Big Sandy region of Appalachia may sometimes be of unhealthy quality. This, in itself, warrants further study. Therefore, efforts should be made to more systematically investigate, model and compare domestic well water quality in areas of the Big Sandy less impacted by mining with more impacted areas. To this end, more collaboration between states as well as research and citizen groups in data sharing is warranted. Already, there has been some productive discussion across borders between citizen groups and research teams to share data and GIS techniques. But more formal collaborations and working partnerships are required to develop more systematic comparisons and controls in order to isolate and test the effects coal slurry waste and other coal mine activities on well water. More systematic data collection, comparisons and controls will allow us to better determine whether or not poor well water quality is associated with active coal mining, an association that many in the region assert to be true, but that has so far been difficult to "prove." Systematic analysis through collaborative development of geocoding and GIS methods, such as the additional overlay of deep-mine injection points and surface and underground mine activities with well water data, will allow us to more objectively determine the association between coal mine activities and possible impacts on private drinking water within the Big Sandy Region. Future research would be greatly facilitated by interested university or private sector scientists or interested citizen groups seeking a collaborative partnership with any of several state agencies, such as the Kentucky Geological Survey, which maintains the Kentucky Groundwater Database Repository, the Kentucky Division of Water, or the University of Kentucky's Kentucky Water Resources Research Institute. This is in accord with emerging collaborative decision-making models that are designed to monitor and protect water resources.

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Building a Centralized Database for Kentucky Fishes: Progress and Future Applications

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ABSTRACT

Kentucky has a long history of ichthyological collection and study. Voucher specimens are available for collections dating back to 1870 and are housed in various museums and research collections in the United States. In this paper we present a descriptive overview and the current status of a project aimed at building a centralized database for vouchered records of Kentucky fishes. To date, we have entered over 51,000 records into the database. Each of these entries contains at least three vital pieces of information: species identification, georeferenced locality, and time of collection. With increasing recognition of the potential value of museum-based data in biological, ecological and conservation studies such a centralized database will serve as an important scientific resource for the study of Kentucky fishes.

KEY WORDS: fishes, database, Kentucky, biodiversity, historical, museum

INTRODUCTION

Ichthyological investigations in Kentucky date back to the earliest scientific documentation of the state's fish fauna in the "Ichthyologia Ohiensis" (Rafinesque 1820). In this work, Rafinesque provided natural history and descriptive information for fishes mostly from the Ohio River near the falls at Louisville. Only a small number of collections are known prior to 1820 and include anecdotal accounts of larger species, e.g., from the Ohio River (Pearson and Krumholz 1984). Woolman (1892) conducted a more comprehensive survey of Kentucky fishes and documented statewide historical distributional information prior to widespread anthropogenic influence. More recent literature on Kentucky fishes includes a distributional catalogue (Evermann 1918), state fish book (Clay 1975), a species checklist (Burr 1980), an Ohio River status and distribution update (Pearson and Krumholz 1984) and a statewide distributional atlas (Burr and Warren 1986). The Kentucky State Nature Preserves Commission, the Kentucky Division of Water, and the Kentucky Department of Fish and Wildlife Resources regularly conduct surveys and produce reports which contribute to the understanding of fish distributions and the status of streams and rivers in the state. Other notable contributions were made by Minor E. Clark, William R. Turner, and those associated with the Ohio River Valley Water Sanitation Committee who provided detailed descriptions of watersheds in the state, pre-impoundment studies, and detailed documentation of the Ohio River ichthyofauna, respectively.

The most comprehensive assessment of Kentucky fish distributions was provided by Burr and Warren (1986). They presented a detailed discussion of physiographic and hydrographic features in Kentucky, species accounts that included point locality range maps, narrative descriptions of distribution, systematics, habitat, and a recommended conservation status. The distributional data in the atlas span from 1818 to 1985 and include records from all major museum holdings in the United States as well as records from personal collecting efforts by the authors. The range maps were fashioned by hand and no computerized records were created during the project. Burr and Warren (1986) is still the principle reference for Kentucky fishes. However, taxonomic revisions, descriptions of new species, and numerous new collection records since 1986 warrant updating of the distributional data.

In recent years there has been increasing interest in museum-based informatics (Graham et al. 2004). Natural history collections offer a wealth of temporal and geographic data tied to animal and plant collections. Developments in computing and analytical tools, especially geospatial technologies, are making these data more relevant and useful. As a result, many of the records used in producing

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the distributional atlas (Burr and Warren 1986) now have been electronically cataloged. These data are available by written request to collection managers or, in some cases, via searchable internet databases. Efforts are underway by various museums and institutions to digitize and link databases online – allowing users to perform a single query across multiple databases. One such project for fishes is Fishnet 2 (www.fishnet2.net). This website allows a user to search fish records across 29 institutional databases with a single query, and results can be exported in a variety of formats for subsequent processing. However, this system has limitations: 1) only a relatively small percentage of the data are georeferenced, 2) there is no consistent schemata to the exported data, and 3) taxonomy rarely is updated for historical records.

In light of the current usage and potential utility of museum-based information for the study, conservation, and management of Kentucky fishes, we decided to build a centralized database for the state. Because many conservation plans and research projects are implemented within state boundaries, such a database would provide historical and contemporary data that would be readily available for analyses. The database also would include information linking records with museums containing voucher specimen(s). The intention of this project is to enhance research and conservation efforts focused on Kentucky's ichthyofauna.

The objectives of this multi-year, ongoing project are to 1) obtain records of Kentucky fish collections from all major museum and institutional holdings, 2) computerize and georeference those records and merge the data into a single dataset with a standardized information schemata, 3) complete error checking and data cleaning, and 4) update taxonomic and nomenclatural information. We provide an overview of our progress and the current status of the database.

MATERIALS AND METHODS

In 2005, Southern Illinois University at Carbondale (SIUC) acquired the ichthyology collection from the University of Louisville (UL). A major task was undertaken to curate, georeference, and computer catalogue the estimated 13,000 lots from UL. In addition to the UL project, we also began curating, georeferencing, and computerizing backlogged records and collections from SIUC, Kentucky State Nature Preserves Commission, Kentucky Department of Fish and Wildlife Resources, and the Kentucky Division of Water. This initiative provided the impetus to expand our efforts to create a comprehensive and centralized database of Kentucky fish records. This database is hereafter referred to as the Kentucky Fishes Database (KFD).

We estimate that greater than 90% of the vouchered collection records for Kentucky fishes are located in the following museums: California Academy of Sciences (CAS), Cornell University (CU), Illinois Natural History Survey (INHS), University of Kansas (KU), National Museum of Natural History (USNM), Ohio State Museum of Biological Diversity (OSM), Southern Illinois University Carbondale (SIUC), University of Michigan Museum of Zoology (UMMZ), and Tulane University (TU). We requested or downloaded all Kentucky fish records from these museums. Many other smaller collections also contain valuable data including Morehead State University (MoSU) and Eastern Kentucky University (EKU). We acquired paper copies of select records from MoSU and incorporated them into the database. The research collection at Murray State University had earlier been transferred and catalogued into the SIUC system.

Records received from museums were in various processed states. For example, some records were fully georeferenced and needed only to be converted to decimal degree format and fitted to the KFD schemata – whereas others were not georeferenced and contained numerous taxonomic and geographical errors. Most of the datasets, however, contained good descriptive information on localities but were not georeferenced and had out-dated taxonomy.

For each record processed, we georeferenced the site in decimal degree format using the North American Datum of 1983, updated taxonomic and nomenclatural information, and standardized the data schemata. Formats for data and headers were specifically chosen for quick incorporation into a geographical

Table 1. Contributing museums and respective number of records provided. Records from SIUC include the ~13,000 lots incorporated from the University of Louisville.

Museum	Contributed Vouchered Records
California Academy of Sciences	246
Cornell University	1051
Illinois Natural History Survey	4100
University of Kansas	598
Morehead State University	1261
National Museum of Natural History	908
Ohio Museum of Biological Diversity	2108
Southern Illinois University Carbondale	36,724
Tulane University	875
University of Michigan Museum of Zoology	3342
TOTAL	51,213

information system (GIS), which played a key role in data editing and enrichment. Ultimately, each record contains three vital dimensions: specimen identification, georeferenced collection locality, and time of collection. All records in the KFD are vouchered records. The incorporation of only vouchered records was intentional and assures that each entry is backed by a physical specimen; this allows the verification of species identification, use in research projects, and increases data integrity.

RESULTS AND DISCUSSION

In 2005, the SIUC collection contained about 10,000 computerized records for Kentucky. As of early 2008, after the incorporation of the UL collection and processing of backlogged collections from state agencies, that number exceeded 36,000. At the end of 2008, SIUC will have more than 40,000 records of Kentucky fishes dating from the 1920s. We have voucher records for every extant species in Kentucky. SIUC's records – including those incorporated from UL – account for about 70% of the records in the KFD.

As of early 2008, the KFD contained over 51,000 records (Table 1). Excluding SIUC, the largest contributors of Kentucky records were INHS and UMMZ with 4100 and 3100 contributed records, respectively. The CAS and the USNM contained fewer records, but housed the majority of the A.J. Woolman and P.H. Kirsch collections, which are of historical value. Ultimately, we expect the KFD to exceed 60,000 records. We are currently processing additional records from the University of Florida, University of Tennessee, Academy of Natural Sciences of Philadelphia, University of Alabama, Field Museum of Natural History, and the Harvard Museum of Comparative Zoology. In addition, we continue to process records from state agencies in Kentucky and from our own collection efforts.

The KFD includes records dating from the 1870s. Woolman's collections represent the earliest comprehensive surveys included in the database. Fewer than 20 pre-Woolman records, from unnamed collectors, exist for the entire dataset. The spatiotemporal coverage of the KFD (Figure 1) is statewide for selected time periods but most comprehensive for the 1970–1990 decades. The majority of vouchered specimens were added after 1950 with most specimens collected since 1980. Despite over 100 years of collection efforts, there are still some regions of the state lacking adequate coverage. Historically, the Nolin River drainage has lacked basic survey work. Likewise, additional collection efforts in the lower Green River, Tug Fork, Mississippi River, and direct Ohio River tributaries would permit a more complete documentation of Kentucky's ichthyofauna.

A consistent data schemata is being applied to all KFD records (Table 2). We encourage additional data enriching by incorporation of comments from collector's field notes, published papers, and reports when available. Such ancillary information (e.g., general comments on habitat, unusual observations, collecting methods/techniques, information on species not vouchered, designation as type specimens, etc.) can greatly enrich the point data. To date, we have georeferenced all records acquired (Table 1), but have not completed the totality of editing and enriching the data. Updating taxonomy from historical collections has proven to be time consuming, and we have made only moderate progress. For newer collections and especially those housed at SIUC we have completed most of the taxonomic updating.

Nomenclatural and taxonomic issues are being addressed using several methods. For species with contrasting biogeographies we are able to clean the data within a spatial

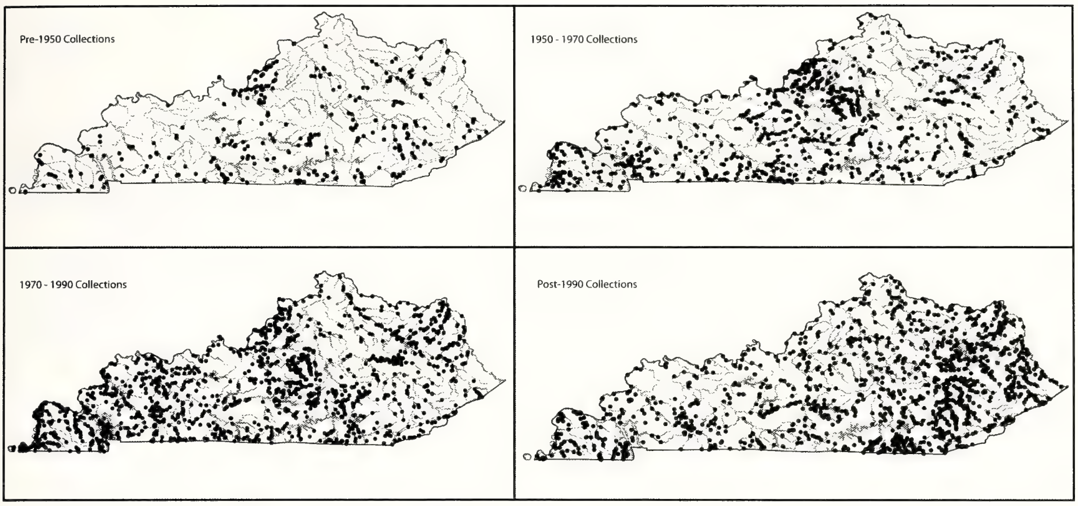

Figure 1. Overview by time periods of collection records currently contained in the Kentucky Fish Database.

format. For example, the *Etheostoma spectabile* (Agassiz), *E. simoterum* (Cope), *E. virgatum* (Jordan), and *Notropis rubellus* (Agassiz) complexes, each containing species with allopatric ranges are edited by plotting the target data within a GIS and assigning correct names to points using spatial-selection methods. In contrast, much editing of older names is being done in a standard database format. Despite extensive cleaning, we continue to find errors within the database. Based on past experiences, we estimate that perhaps 5–7% of the entries contain errors of some nature (e.g., out-dated taxonomy, misspellings, etc.) with 1–2% containing errors that seriously compromise the integrity of the data (e.g., incorrect locality information or misidentification). Focus on the three vital dimensions (identification, georeferenced localities, and time of collection) for each record is required to ensure high quality datasets.

Table 2. Data schemata for database with examples of included information.

Field Name	Information
Museum	SIUC
CatNum	64,942
Species	*Etheostoma derivativum*
Latitude	36.8822
Longitude	−87.1111
Waterbody	Whippoorwill Creek
Drainage	Red-Cumberland River
County	Todd
State	Kentucky
Day	19
Month	April
Year	2007
Collectors	RL Hopkins, LJ Fisk
Locality	at SR 106 bridge; 0.5 mi S of Claymour, KY
Notes	riffle area now inundated due to fallen tree

The potential applications of the KFD data are multifold. Museum-based data can be integrated with other data types to address a series of questions ranging from conservation research to the study of ecological and evolutionary processes. Other applications include spatial analysis of biodiversity, the study and modeling of species distributions, analysis of range shifts, planning and developing future field projects, identification of potential areas for reintroductions, etc. To illustrate the utility of the KFD, we have completed a cursory spatiotemporal analysis of the distribution of the Trout-Perch (*Percopsis omiscomaycus* Walbaum) in Kentucky (Figure 2). Some localities for this species included in Burr and Warren (1986) are not shown on the map (Figure 2) because the KFD is restricted to vouchered records. In this case, the voucher for a collection of Trout-Perch Welter (1938) in North Fork Triplett Creek (Licking River drainage) reportedly deposited at MoSU could not be located.

The overall pattern that emerges (Figure 2) is the absence of Trout-Perch in recent collections from the western portions of its range. In contrast, eastern populations in Little Sandy River, Levisa Fork, Tygarts

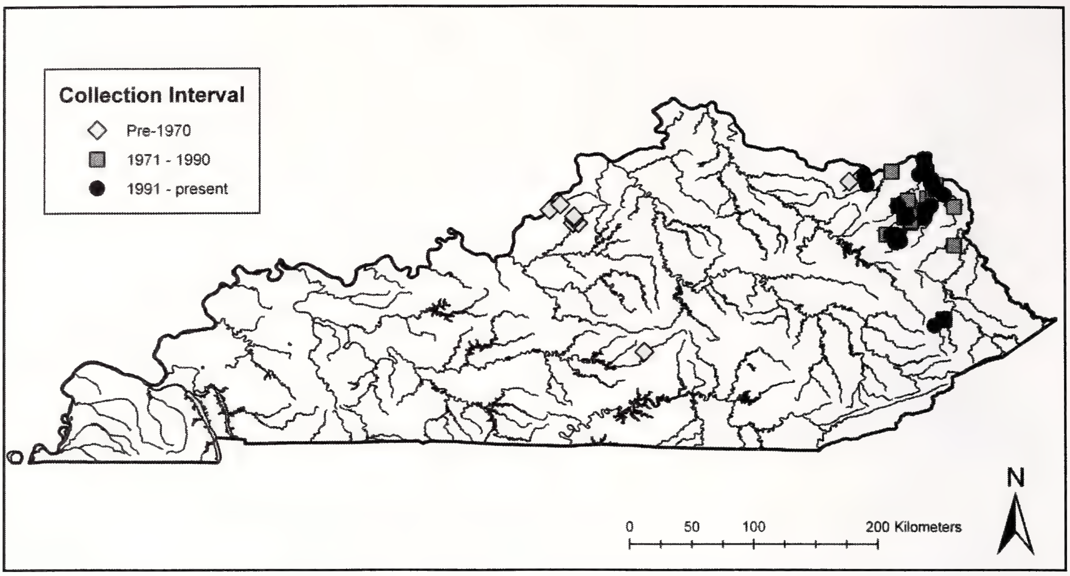

Figure 2. Spatiotemporal map of Trout-Perch (*Percopsis omiscomaycus*) records in Kentucky.

Creek, and Kinniconick Creek seem stable, although, no recent specimens have been captured from the East Fork of the Little Sandy River or Little Blaine Creek (Big Sandy River drainage). The apparent disappearance of this species in western areas warrants further research investigating the causes of its range contraction in Kentucky.

CONCLUSIONS

Since building the database we have been able to respond to questions about the Burr and Warren maps much more effectively. We are not only able to provide locality information, but also the time of collection, holding museum, a list of other species captured at the site, etc. Upon request, we can produce maps and spreadsheets containing the aforementioned information. It is evident that such a database is a valuable tool for researchers and will increase the use of museum and research collections in ichthyological, ecological, and conservation studies.

Furthermore, as we begin to mine the data we are finding many noteworthy records for Kentucky fishes. We have evidence of range expansions and contractions and of new drainage records for several species. The collection from UL has provided a wealth of additional historical information for Kentucky fishes. In addition, there have been multiple recent collections of rare fishes – an encouraging sign. We are preparing a manuscript providing an overview of some of the more noteworthy records stemming from the KFD project. We encourage museums of all sizes to georeference and computerize their fish collection records, so that they be more accessible for use in conservation planning and the study of evolutionary and environmental processes.

ACKNOWLEDGEMENTS

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Mössbauer Study of the Half-Metallic Ferromagnet $Fe_{1-x}Co_xSi$

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ABSTRACT

Mössbauer spectroscopy and X-ray diffraction measurements were performed to probe the magnetic properties of $Fe_{1-x}Co_xSi$ ($0 \leq x \leq 0.8$) compounds at room temperature. X-ray diffraction measurements showed that the crystal structures of the synthesized samples existed as a single phase for all concentrations. Fittings of the Mössbauer spectra revealed that the family of $Fe_{1-x}Co_xSi$ are weakly ferromagnetic for $0.1 \leq x < 0.5$ and completely lose their magnetic properties when $x \geq 0.5$.

KEY WORDS: Mössbauer, isomer shift, quadrupole splitting, half-metal, ferromagnets

INTRODUCTION

In 1983, de Groot et al. discovered that half-metallic ferromagnets existed when only charge carriers of one spin direction contributed to the conduction band (de Groot et al. 1983). This implies that there is a band gap at the Fermi level for one spin direction. Following their discovery, there has been enormous interest in half-metals, as they form the basis of the new field appropriately termed spintronics. The bands calculation of the compound $Fe_{0.8}Co_{0.2}Si$ clearly shows the Fermi level in a gap of the minority band (Figure 1) (Guevara et al. 2004). These compounds had been studied even before the discovery of half-metalicity (Wertheim et al. 1966; Beille et al. 1981).

The $Fe_{1-x}Co_xSi$ alloys form disordered solid solutions of cubic structure B20 at all concentrations (Figure 2) (Racu et al. 2007; Fanciulli et al. 1996). The $Fe_{1-x}Co_xSi$ compound is remarkable in that when $x = 1$, CoSi is diamagnetic and when $x = 0$, FeSi is paramagnetic; however, there is a concentration range of x where the $Fe_{1-x}Co_xSi$ alloys are magnetically ordered. The appearance of the ferromagnetic order is due to the interaction between the moments of the cobalt and iron atoms. The range over which these alloys are supposed to be ferromagnetic is not consistent in the literature. It has been reported that the ferromagnetic range is $0.05 < x < 0.95$ (Fanciulli et al. 1996), and when $x = 0.5$, the alloys were found to be non-magnetic (Wertheim et al. 1966). Furthermore, for $x > 0.25$, it is reported that the system starts to segregate Co from Fe atoms leading to the disappearance of half metallicity and to a decrease of the magnetic moment (Guevara et al. 2004).

To determine the range over which these $Fe_{1-x}Co_xSi$ alloys are magnetically ordered, alloys were synthesized in the range of $0 \leq x < 0.8$. X-ray diffraction measurements were used to determine the crystal structure of the alloys and Mössbauer spectroscopy measurements were employed to search for the appearance or disappearance of magnetic order in these alloys.

METHODS

The alloys were prepared using the solid state method. Twenty millimoles of stoichiometric proportions of pure Fe, Co, and Si were mixed, pulverized and cooked at 1100°C for 15 hr under the flow of argon. The samples were allowed to slow cool after which they were pulverized and X-ray diffraction measurements were taken at room temperature. A Phillips X'Pert diffractrometer was used to collect the X-ray crystal structure data.

Mössbauer absorption measurements were taken in excess of 1,000,000 counts with about 5 mCi of ^{57}Co-embedded in a rhodium matrix source. The data were taken in the sinusoidal mode and fitted with the commercially available Recoil fitting routine. The Mössbauer system (MVT 1000) used to collect data was made by the German Wessel Company.

RESULTS AND DISCUSSION

The X-ray diffraction patterns of all of the $Fe_{1-x}Co_xSi$ ($0 \leq x \leq 0.8$) samples showed a single phase and are iso-structural with the

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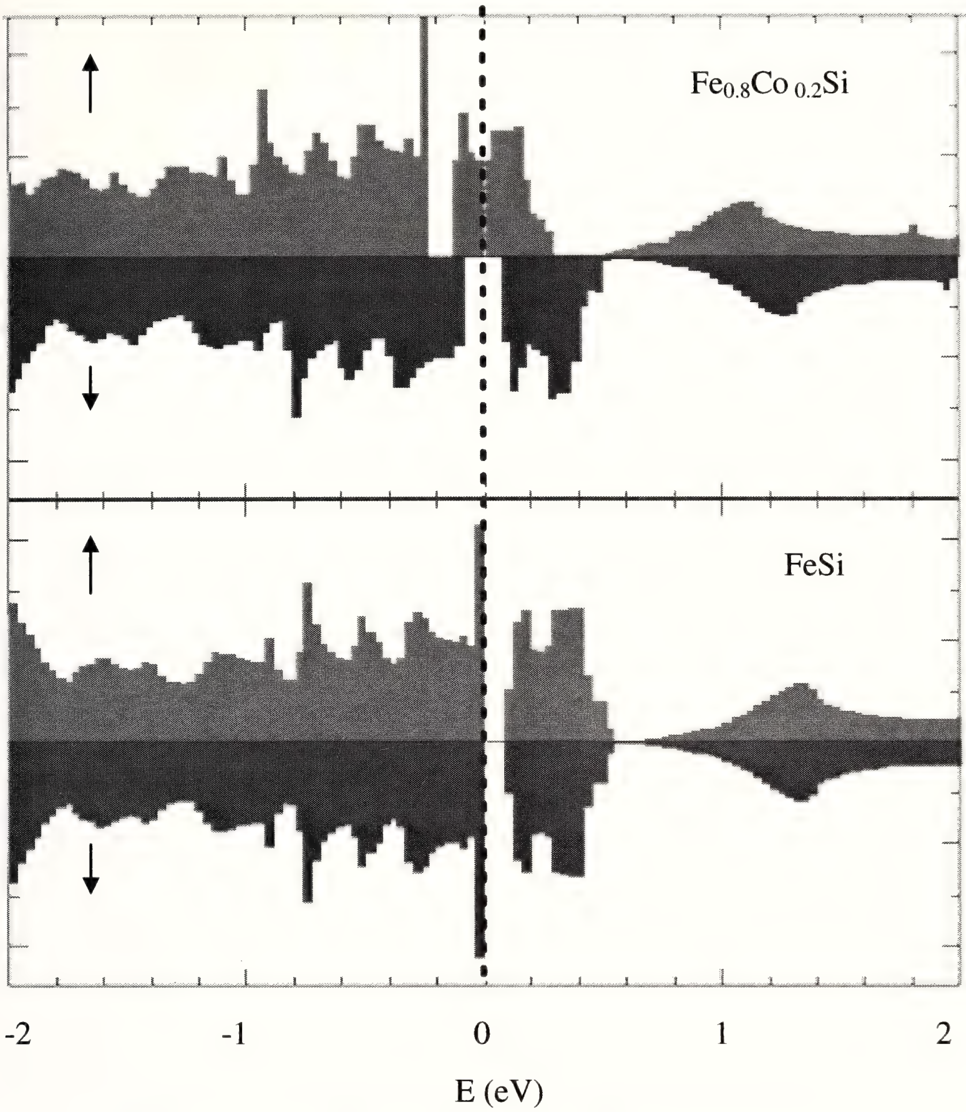

Figure 1. Density of states (arbitrary units) of $Fe_{0.8}Co_{0.2}Si$ and FeSi.

parent compounds FeSi and CoSi (Figure 3). The Fe local symmetry is trigonal, with one Si nearest neighbor at 0.229 nm, three Si second nearest neighbors at 0.236 nm and three Si third nearest neighbors at 0.253 nm (Figure 2) (Fanciulli et al. 1996). As mentioned earlier, these alloys possessed the B20 cubic structure which allowed for the existence of nuclear electric quadrupole and anisotropic magnetic dipole interactions. These effects were manifested in the resulted Mössbauer spectra of all the samples by showing a quadrupole splitting doublet (Figure 4). The Mössbauer results clearly showed that $Fe_{0.9}Co_{0.1}Si$ and $Fe_{0.8}Co_{0.2}Si$ were magnetic and had hyperfine magnetic fields (hmf) of 18.7 and 31.5 Tesla respectively which were referenced to metal iron with an hmf of 33 Tesla. The samples $Fe_{0.5}Co_{0.5}Si$ and $Fe_{0.2}Co_{0.8}Si$ showed no magnetic splitting (Figure 4).

All of the data were fitted with only one doublet and one sextet for x = 0.1 and x = 0.2 samples as they showed magnetic splitting (Table 1). The data could be fitted with two sets of doublets, representing the different sites that the iron occupies, however, these samples were polycrystalline (powders), and

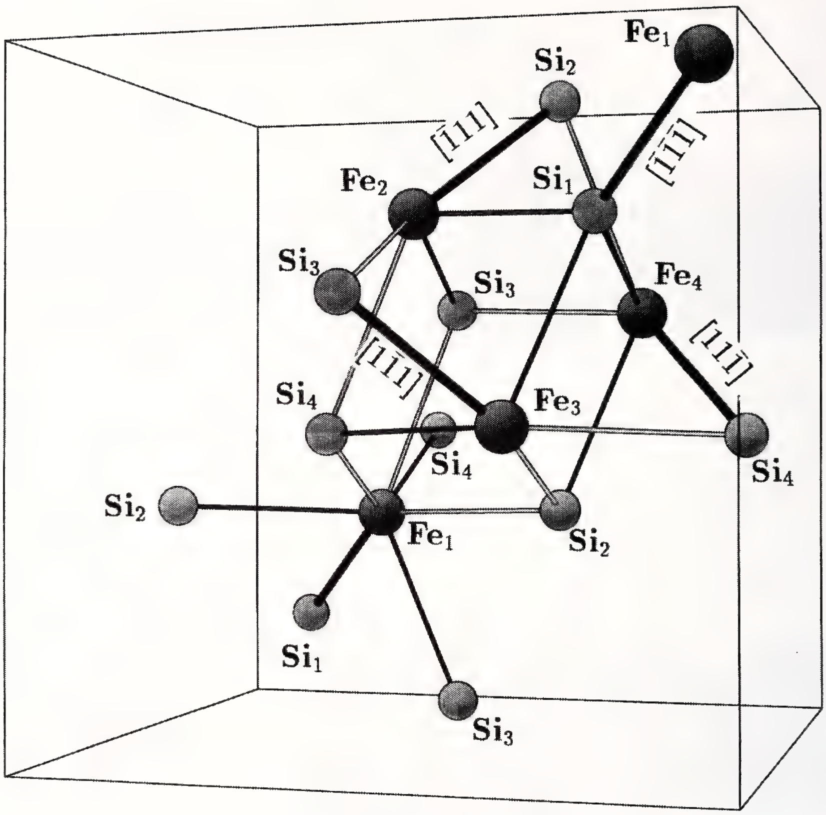

Figure 2. The B20 structure of the e-FeSi compound. The numbers 1, 2, 3, and 4 denote the atoms at different positions.

any differences are not resolved in the Mössbauer spectra hence only averages are reported.

The resulted isomer shift values were constant (within the error bars), which suggested that the crystal configuration remained the same as more cobalt atoms replaced iron atoms (Figure 5). It also illustrated that the cobalt and iron atoms were equivalent. On occasions when there was a small variation in the isomer shift values, it usually was due to the charge imbalance that was screened by electrons on the iron atom itself.

On the other hand, a decrease of the quadrupole splitting values was observed as the cobalt concentration was increased (Figure 6), which was consistent with the assumption that the electric field gradient due to distant charges was suitably modified by antishielding factors. As iron atoms were added to the CoSi compound, the iron contribution was increased due to the presence of more nearest iron neighbors; however, the observed increase was not linear as there was an abrupt change in the quadrupole splitting around $x = 0.2$ (Figure 6).

The line width was observed to experience a slight increase from 0.157 mm/sec at $x = 0.8$ to 0.189 mm/sec for $x = 0.2$ when cobalt was substituted for iron (Figure 7). This trend seemed reasonable since as cobalt replaced iron there would be fewer iron-iron neighbors. The result was consistent with that reported in the literature (Preston et al. 1966). The line width of the reference metal iron was 0.1478 ± 0.0036 mm/sec.

Finally, the measured hyperfine magnetic field increased as x increased, peaked around $x = 0.2$ and vanished for $x \geq 0.5$ (Table 1), which was consistent with reported increase in the magnetic moment in the range of $0.08 \leq x \leq 0.25$ (Chernikov et al. 1997).

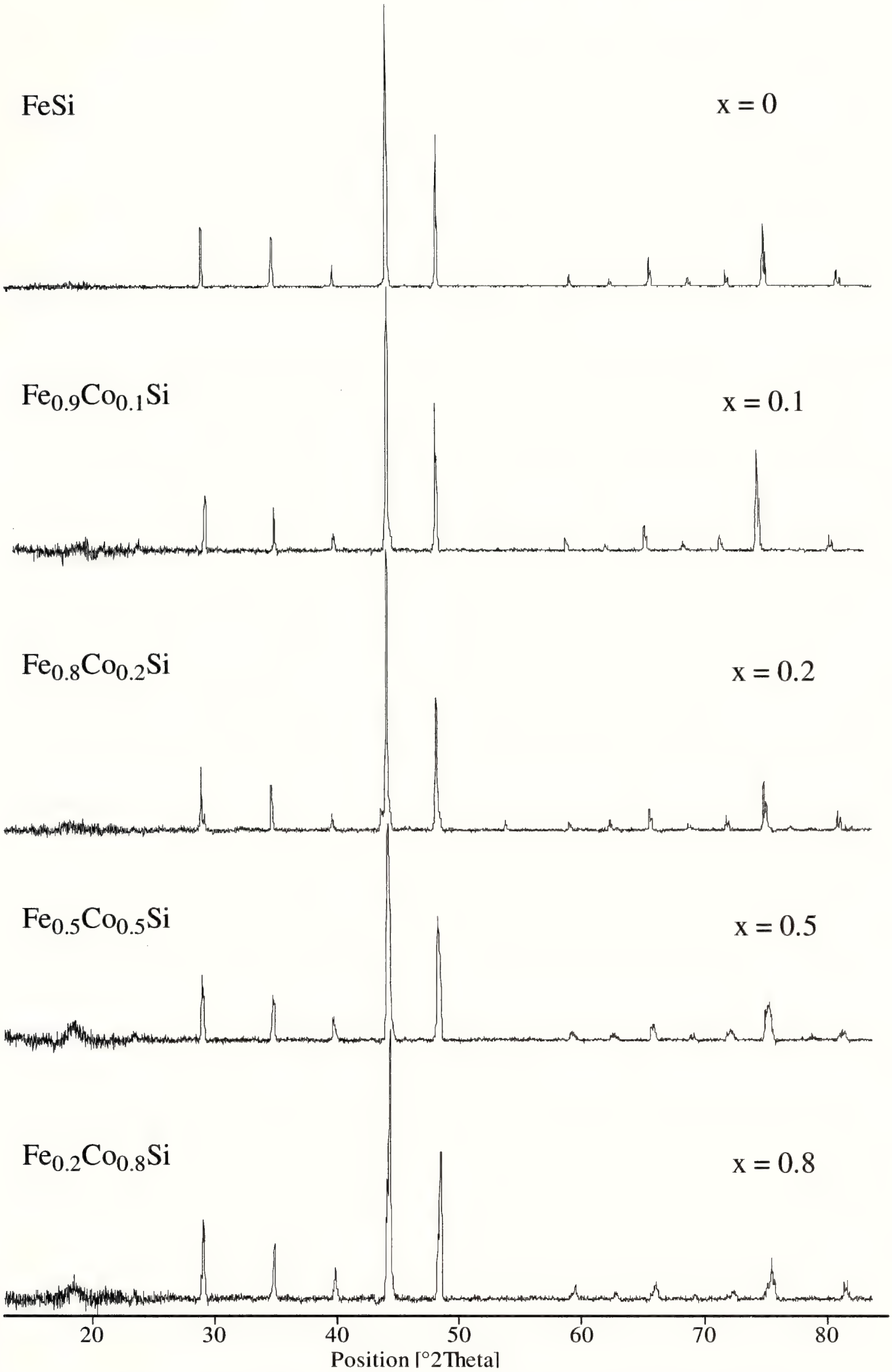

Figure 3. X-ray diffraction patterns of $Fe_{1-x}Co_xSi$(x=0, 0.1, 0.2, 0.5, and 0.8).

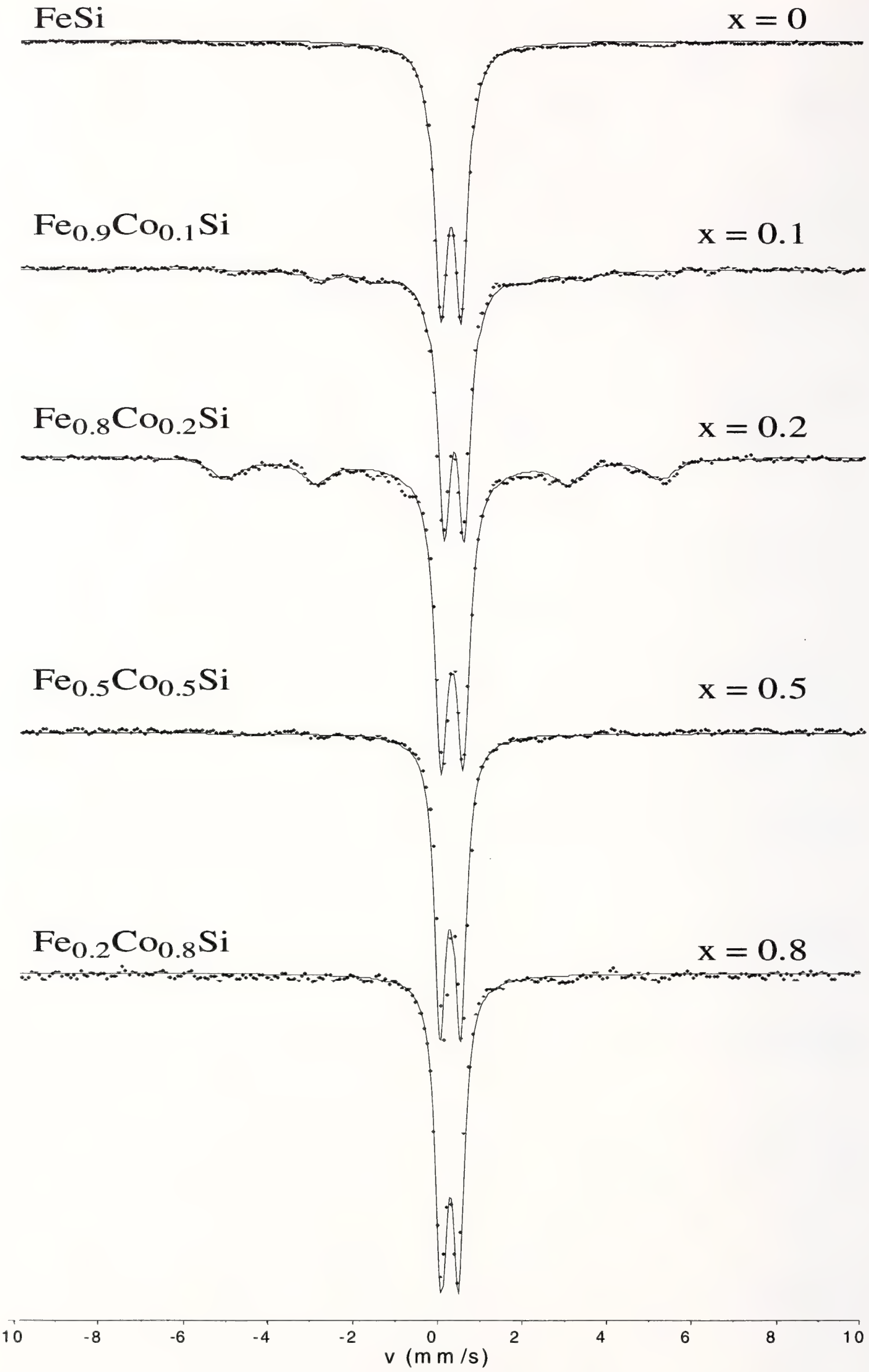

Figure 4. Mössbauer data fitted with one doublet for x = 0, 0.5. and 0.8 samples or with a doublet and a sextet for x = 0.1 and 0.2 samples.

Table 1. Summary of the results of the fitting routine (Recoil). I.S. is the isomer shift, Δ is the quadrupole splitting, and hmf is the hyperfine magnetic field.

Sample	Linewidth (mm/sec)	I.S. (mm/s)	Δ (mm/s)	hmf (mm/s)	% Site Population
Iron (Ref) - Sextet	0.148 ± 0.004	0	0	2.238 ± 0.001	100
FeSi - Doublet	0.184 ± 0.002	0.276 ± 0.001	0.485 ± 0.001		100
$Fe_{0.9}Co_{0.1}Si$ - Doublet	0.185 ± 0.005	0.273 ± 0.002	0.471 ± 0.003		84
$Fe_{0.9}Co_{0.1}Si$ - Sextet		0.172 ± 0.044	0.005 ± 0.041	1.268 ± 0.028	16
$Fe_{0.8}Co_{0.2}Si$ - Doulet	0.189 ± 0.005	0.278 ± 0.002	0.489 ± 0.003		62
$Fe_{0.8}Co_{0.2}Si$ - Sextet		0.048 ± 0.022	0.012 ± 0.021	2.136 ± 0.012	38
$Fe_{0.5}Co_{0.5}Si$ - Doublet	0.162 ± 0.003	0.280 ± 0.002	0.456 ± 0.002		100
$Fe_{0.2}Co_{0.8}Si$ - Doublet	0.157 ± 0.006	0.278 ± 0.003	0.403 ± 0.004		100

CONCLUSIONS

The synthesis of the $Fe_{1-x}Co_xSi$ resulted in single phased alloys for all concentrations. $Fe_{0.8}Co_{0.2}Si$ exhibited a consistent x-ray diffraction pattern as found in the literature. Mössbauer spectroscopy results showed that $Fe_{0.8}Co_{0.2}Si$ is magnetic and had an hmf of about 30.98 Tesla. When $x \geq 0.5$, the samples lost their magnetic ordering as illustrated by the absence of magnetic splitting in the Mössbauer spectra of $Fe_{0.5}Co_{0.5}Si$ and $Fe_{0.2}Co_{0.8}Si$ alloys.

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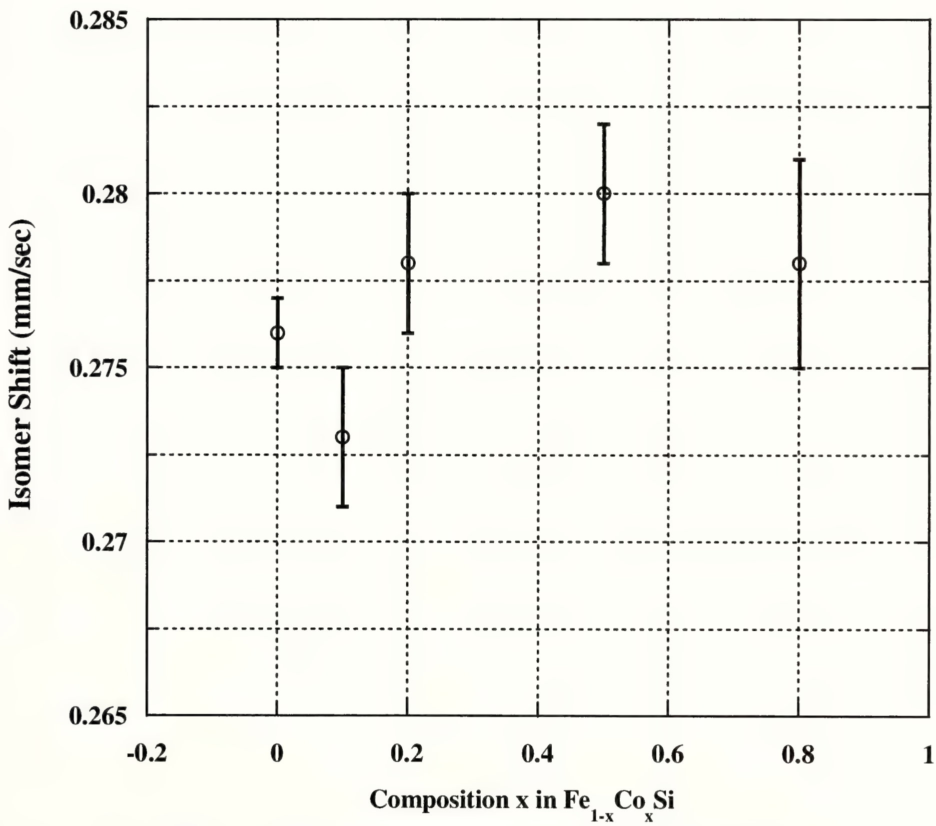

Figure 5. Isomer Shift of $Fe_{1-x}Co_xSi$ (x=0, 0.1, 0.2, 0.5, and 0.8).

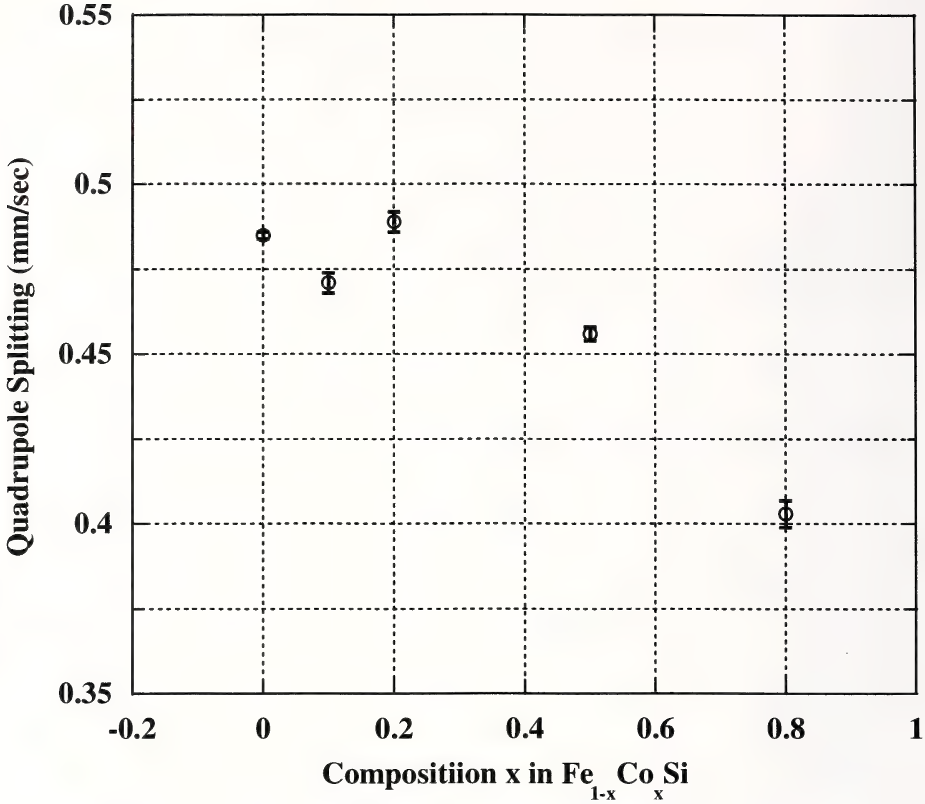

Figure 6. Quadrupole Splitting of $Fe_{1-x}Co_xSi$ (x=0, 0.1, 0.2, 0.5, and 0.8).

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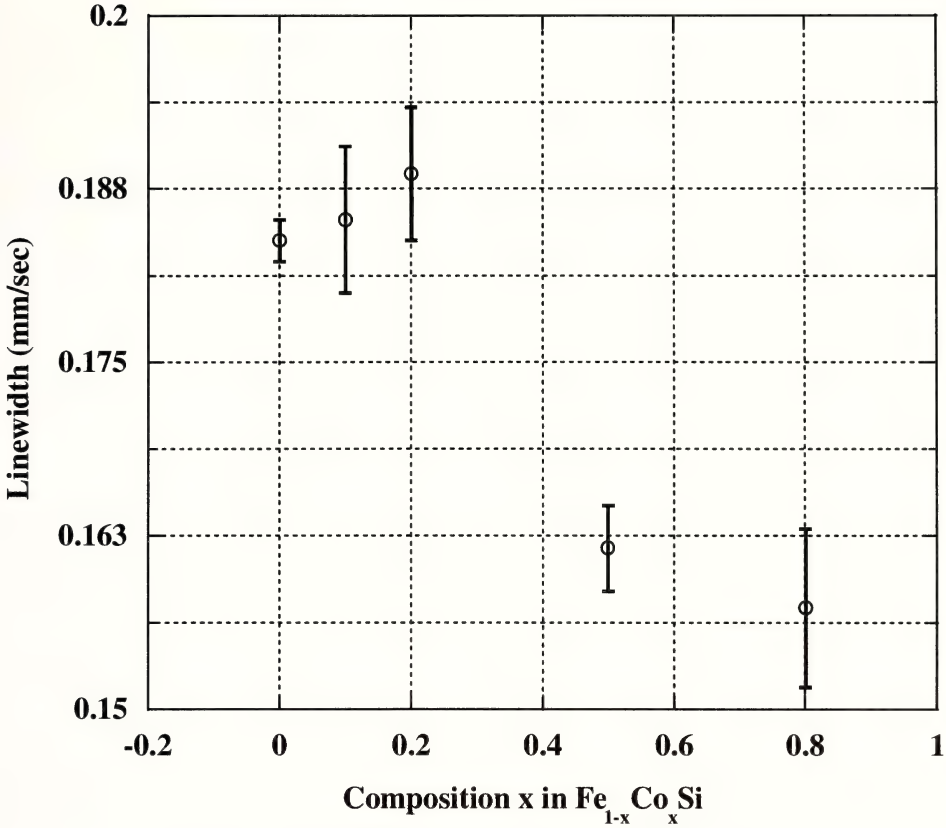

Figure 7. Line-width of $Fe_{1-x}Co_xSi$ (x=0, 0.1, 0.2, 0.5, and 0.8).

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Reproductive Biology of the Northern Madtom, *Noturus stigmosus* (Siluriformes: Ictaluridae) from the Licking River, Kentucky

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ABSTRACT

The reproductive biology of the northern madtom, *Noturus stigmosus* Taylor, was examined in the Licking River, Bath and Rowan counties, Kentucky from 2001–2006. Gonadosomatic indices suggest a discrete spawning event in mid-summer, with sexual maturity reached at approximately 60 mm standard length (SL). Clutch size was estimated at 70–110 eggs per female based on examination of mature ovaries. Males and females come into reproductive condition in early summer and exhibit secondary sexual dimorphism typical of madtoms. Four nests were discovered in early to mid-July in water 23–25°C. All nests were in cavities under large slabrocks in a raceway with moderate current (0.36–0.69 m sec^{-2}) above a large riffle. Nests contained 40–87 eggs or embryos; no guardian males were found with any nest. Eggs reared in the lab at 21–22°C hatched about 13 days after fertilization, which is the longest reported for madtoms. Embryonic and larval development were similar to those of other madtoms. Hatchlings were 8.1–9.3 mm total length (TL). By 10 days, the yolk sacs were absorbed, and young (now 15.4–15.7 mm TL) had acquired pigmentation diagnostic for the species. Approximately one month after hatching (at ~20 mm SL), young moved downstream from the raceway into a large riffle.

KEY WORDS: Madtom, *Noturus*, reproductive biology, Ictaluridae, life history

INTRODUCTION

The northern madtom, *Noturus stigmosus* Taylor, is a small catfish (<100 mm total length) that is widespread, but uncommon and localized in large streams in the Ohio River and Great Lakes basins. In Kentucky a relatively large population is present in the Licking River at Moore's Ferry, about 20 stream km downstream of Cave Run Lake dam. There, the northern madtom, along with two other madtoms, the stonecat, *Noturus flavus* Rafinesque, and the brindled madtom, *Noturus miurus* Jordan, occupy a large riffle-raceway complex. The species is of "special concern" in Kentucky (KSNPC 2000) and protected or extirpated in most other states in its range (Scheibly 2003).

Life-history studies and published descriptive notes have documented the reproductive biology of most madtom species (Burr and Stoeckel 1999), but little has been reported for *N. stigmosus*. Taylor (1969) provided anecdotal reports of nests, all but one from anthropogenic substrates, from the Huron River, Michigan, but provided few details. The specimens collected by Taylor were reexamined by Thomas and Burr (2004), and the data were reported in their description of *Noturus gladiator* Thomas and Burr. Several nests of *N. stigmosus* were recently documented in Lake St. Clair, Ontario, under artificial substrates designed to study the reproductive biology of the round goby, *Neogobius melanostomus* Pallas (MacInnis 1998). Thus, few recent data for nests in streams or nests using natural materials are available, and no information has been

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published concerning embryonic or larval development.

The objective of this study was to describe the reproductive and nesting biology and early life-history of *N. stigmosus* based on field observations of nests and specimens collected from the Licking River at Moore's Ferry, Kentucky, and embryos and larvae reared in the lab.

METHODS

Reproductive Traits

Methods for determining reproductive characteristics of *N. stigmosus* followed Mayden and Burr (1981) and Stoeckel and Burr (1999). Seasonality of reproduction and age at sexual maturity were determined from gonadosomatic indices (GSI), microscopic examination of gonads, and presence of secondary sexual characteristics using specimens from Moore's Ferry previously catalogued in the Morehead State University Museum (MOSU) or collected during this study. Gonadosomatic indices were calculated as gonad weight divided by adjusted body weight ×1000; adjusted body weight equaled total body weight minus weight of stomach, intestine, liver, and gonads. Terminology and classification of gonad and ova maturity follow that used by Burr and Mayden (1982a) and Stoeckel and Burr (1999). Counts of mature oocytes were used to estimate female fecundity. Age classifications were based on length-frequency analyses (Scheibly 2003) following Everhart and Youngs (1981) and Macdonald (1987).

Surveys for Nests

The upper end of the riffle and associated raceway at Moore's Ferry in the Licking River, Bath and Rowan counties, Kentucky, were surveyed for nests from late June to early August in 2001, 2002, 2005, and 2006. Rocks and other substrates potentially harboring nests were checked while snorkeling or wading. Total survey time was 28.5 person-hours. Descriptive data of discovered nests and associated habitat were recorded. Flow was measured with a Swoffer 3000-C140 flowmeter or by timing a floating object moving over the nest. Egg masses discovered were removed from the river and brought to the lab at MOSU to study development and confirm identification.

Early life History Description

Egg masses brought to the lab were kept in river water at 21–22°C in a 1-L jar and vigorously aerated with an airstone. Ages of egg masses at the start of lab culture were estimated based on published descriptions of *N. exilis* Nelson (Mayden and Burr 1981) and *N. nocturnus* Jordan and Gilbert (Burr and Mayden 1982b). Descriptions of developing embryos and larvae were made at approximately 24 hr intervals. Periodically, a few embryos or larvae were fixed in 5% formalin and vouchered in the MOSU collection for further study.

RESULTS

Male Reproductive Cycle

Testes of male *Noturus stigmosus* were similar in structure and appearance to published descriptions of other madtom species (Burr and Mayden 1982a; Burr et al. 1989; Stoeckel and Burr 1999). Mature testes were elongate, opaque white to creamy yellow, with numerous fingerlike projections of various sizes. Immature testes from age class 0 and age class 1 fish were smaller, clear, and had fewer, smaller projections.

Examination showed that testis maturity gradually increased early in growth, with a rapid increase in gonad development after reaching larger sizes. Gonadosomatic indices for these individuals were low in age class 1 individuals (under 60 mm SL) but reached an inflection point and began to rise quickly at 60 mm SL at about twenty-four months of age (Figure 1). Values for adult males were lower in May, began to rise in late June, peaked in mid-July, and dropped in mid-August, remaining low through September (Figure 2).

Reproductive males captured from late June through July showed well-developed secondary sexual characteristics (Figure 3). Specimens had enlarged lips, mandibular adductor muscles, and genital papillae, typical of breeding male madtoms. In addition, reproductive males showed enhanced coloration. The overall hue of the body was orange-yellow with marked areas of pink on the venter and underside of the head, especially

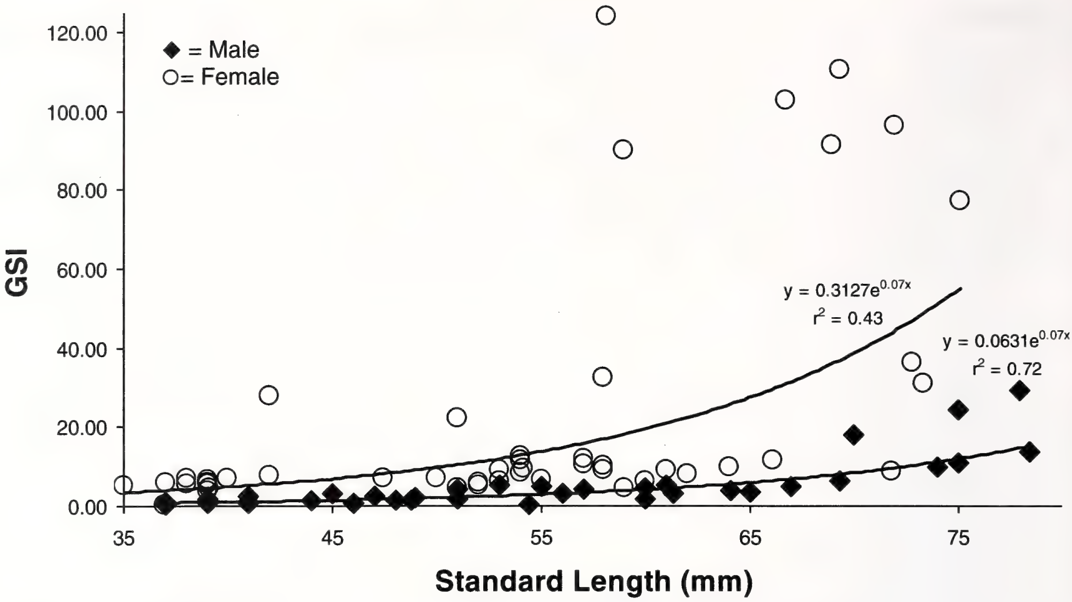

Figure 1. Relationship between gonadosomatic index and standard length for 38 male and 49 female *N. stigmosus* collected from Moore's Ferry.

the lips. The fins, especially the pectorals, had a yellow cast fading to pink or orange in the distal third of the leading edge. No males under 60 mm SL exhibited well-developed secondary sexual characteristics.

Female Reproductive Cycle

Ovaries of female *N. stigmosus* were oblong to kidney shaped, similar to descriptions for other madtom species (Burr and Mayden 1982a; Burr et al. 1989; Stoeckel and Burr 1999). Immature ovaries contained one size class of oocytes, which were small in diameter (0.1–0.5 mm), spherical, and opaque white to creamy yellow. Mature ovaries were larger, with two distinct size classes of oocytes. Resting oocytes were small in diameter (1.2–2.3 mm, mean = 1.2 mm) and similar in color to immature oocytes, while mature oocytes were large (1.0–3.1 mm, mean = 2.2 mm), spherical to polygonal in shape, and yellow-orange to amber.

Females less than 60 mm SL captured during the spawning season showed little gonad development. Gonadosomatic indices were low for the fish in age class 1 (<50 mm SL), but dramatically rose as females reach approximately two years of age (55–58 mm SL) (Figure 1). This relationship was similar to what was seen in males, but the relationship was not as well-defined for females (r^2 = 0.72 and r^2 = 0.43, respectively). Outliers below the fitted curve (i.e., unusually low GSI values) likely represent females that recently spawned. Two smaller individuals (~55 mm SL) that had high GSI values but no distinct mature oocytes in their ovaries may represent females preparing to enter maturity. The seasonal variation of GSI values was similar to that seen in males, although females values may begin to increase earlier in the year. Gonadosomatic index values in reproductive females were high in May, increased slightly in mid-July, fell rapidly in late July, and remained low in autumn (Figure 2).

Females collected during the spawning season exhibited secondary sexual characteristics typical of other species of *Noturus*. Females had swollen genital papillae and distended abdomens, as well as enhanced coloration similar to reproductive males; however, the papillae of females were wider and blunter than those of males and coloration was less intense. Females less than 58 mm SL did not exhibit secondary sexual characteristics during the spawning season.

In eleven reproductively mature females examined, the number of mature oocytes

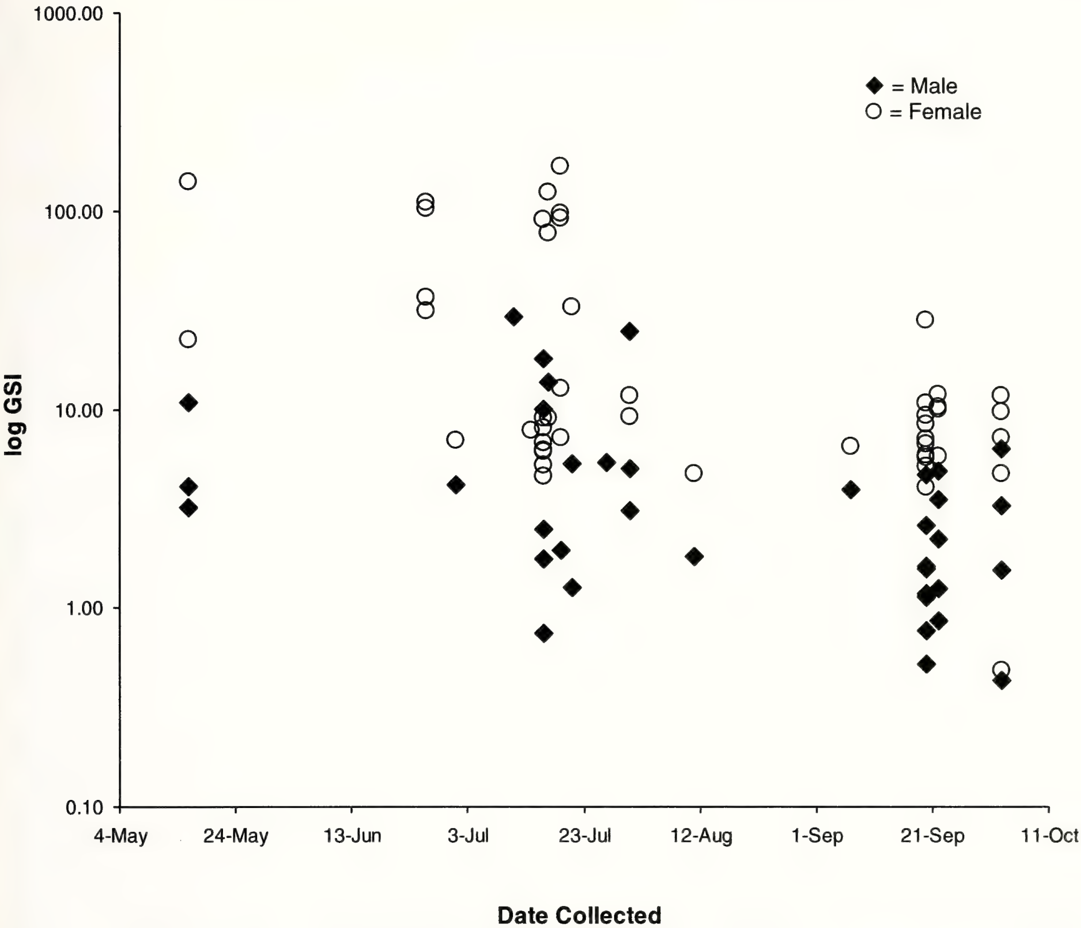

Figure 2. Seasonal variation in gonadosomatic index for 38 male and 49 female *N. stigmosus* collected from Moore's Ferry.

present per female ranged from 34 to 106 (mean = 79.5) (Table 1). Larger, heavier, and older individuals tended to have more mature oocytes per ova, but number of oocytes was not significantly correlated with adjusted weight ($r = 0.426$; $p = 0.191$), age ($r = 0.519$; $p = 0.102$) or length ($r = 0.418$; $p = 0.200$). Lack of statistical significance is attributable to the small sample size of reproductively active females.

Nests

Four likely *N. stigmosus* nests, two of which were confirmed, were found during the 28.5 person-hours of survey (1 nest per 7.1 survey hrs) (Table 2). Nests were found in a raceway 10–40 m above the large riffle in the Licking River at Moore's Ferry. Nests were found between 4 and 12 July in water 23–25°C; Licking River flow was low to moderate (1.4–6.9 m^3 sec) at Cave Run Lake dam, about 14 rkm above Moore's Ferry. The area of the raceway where the nests were located had moderate current (0.36–0.69 m sec^{-1}), a substrate of cobble and gravel, scattered patches of the macrophyte *Potamogeton*, and numerous slabrocks.

All nests were located in 4–7 cm deep cavities under large slabrocks (Table 2). One nest contained about 40 larvae; the other three nests had egg masses with about 55–87 eggs each. One egg mass was attached to live *Potamogeton* roots under the rock; the other eggs masses were not attached to the substrate or nest rock. No guardian adults were found with the nests, and no *N. stigmosus* were found during any snorkeling surveys, although seining the same areas immediately afterwards

Figure 3. Ventral (top) and dorsolateral (bottom) views of *N. stigmosus* collected 17 July 2001. Left, male, 76 mm SL. Right, female, 76 mm SL.

Table 1. Relationship between size, age, ovary weight, and the number of mature oocytes in eleven reproductively mature *Noturus stigmosus* females at Moore's Ferry.

SL (mm)	Adjusted Body Weight (g)[1]	Month of Collection	Age in Years	Weight of Ovaries (g)	Number of Mature Oocytes	GSI[2]
66	4.38	May	3	0.61	106	140
69	5.55	June	3	0.62	86	111
73	5.10	June	3	0.15	71	31
58	3.49	July	2	0.11	34	33
58	5.90	July	2	0.73	76	124
59	4.37	July	2	0.39	81	90
62	4.23	July	2	0.71	82	167
69	5.74	July	3	0.52	83	92
72	5.75	July	3	0.55	66	96
73	6.15	July	3	0.52	96	85
75	6.61	July	3	0.51	98	77

[1] Adjusted body weight is specimen weight after removal of viscera.
[2] Equals weight of ovaries × 1000/adjusted body weight.

revealed *N. stigmosus* to be abundant. Other madtoms, *N. flavus* and *N. miurus*, occasionally were discovered while snorkeling, even though they were much less commonly collected by seining.

One additional madtom nest discovered 26 June 2001 was midway in the riffle. This nest contained a clump of about 98 eggs and an adult male (129 mm SL) *N. flavus* located beneath a large (56 cm × 42 cm × 6 cm) slabrock in strong current. Habitat for this nest was typical of that previously reported for *N. flavus* (Walsh and Burr 1985) and it is assumed to be a *N. flavus* nest. Eggs from this nest appeared to be unfertilized; they were brought into the lab, but did not develop.

Although no guarding males were found with any of the presumed *N. stigmosus* nests, two (4 July and 7 July) could be confidently associated as *N. stigmosus*, because young from the nests were reared to an age when they could be identified. All embryos of the other two nests (12 July) died of fungal infections prior to hatching. These embryos were probably *N. stigmosus*, because only *N. stigmosus* was found (by seining) near the nests, and the clutch size of *N. flavus* is usually much higher (104–306; Walsh and Burr 1985). Based on ages of the larvae or embryos at discovery (Table 2), spawning of the four nests occurred from about 15 June to 10 July.

Early Development

36 hrs (after fertilization). Gastrulation beginning; blastoderm covered about one-half of yolk, with margins visibly thickened into a germ ring. Eggs 3.4–3.6 mm in diameter; yolk mass deep yellow, 3.0–3.1 mm in diameter.

50 hrs. Embryos distinct and elongate, about as long as yolk diameter; brain differentiated into prosencephalon, mesencephalon, and rhombencephalon; rudimentary optic vesicles present; about 20 somites; tail bud apparent; no movement (Figure 4A).

72 hrs. First movements observed – tail occasionally swept laterally.

96 hrs (4 days). Eyes prominent, but lacking pigment; otic vesicle large, clear and subrectangular; two pairs of distinct barbels visible; median fin extends from middorsum,

Table 2. Descriptions of four *Noturus* nests in the Licking River at Moore's Ferry, Rowan-Bath counties, Kentucky. The first two nests (7 July and 4 July) were confidently identified as those of *N. stigmosus*; the two from 12 July were probably those of *N. stigmosus*.

Date	Water Temperature	Depth (cm)	Flow (m/sec)	Nest Rock Size (cm)	Substrate Under Rock	Number of Eggs or Larvae; Age[1]
7 July 2002	25°C	40	0.49	25 × 27 × 8	Fine gravel, sand	40 metalarvae; 2 weeks
4 July 2005	23°C	46	0.69	23 × 32 × 4	Cobble, clamshell	55 eggs, about 96 hrs
12 July 2005	24.5°C	37	0.50	23 × 30 × 9	Sand, gravel, live roots	66 eggs, about 50 hrs
12 July 2005	24.5°C	26	0.36	48 × 41 × 10	Sand, gravel	87 eggs, about 36 hrs

[1] Approximate ages given in hours after fertilization or weeks after hatching.

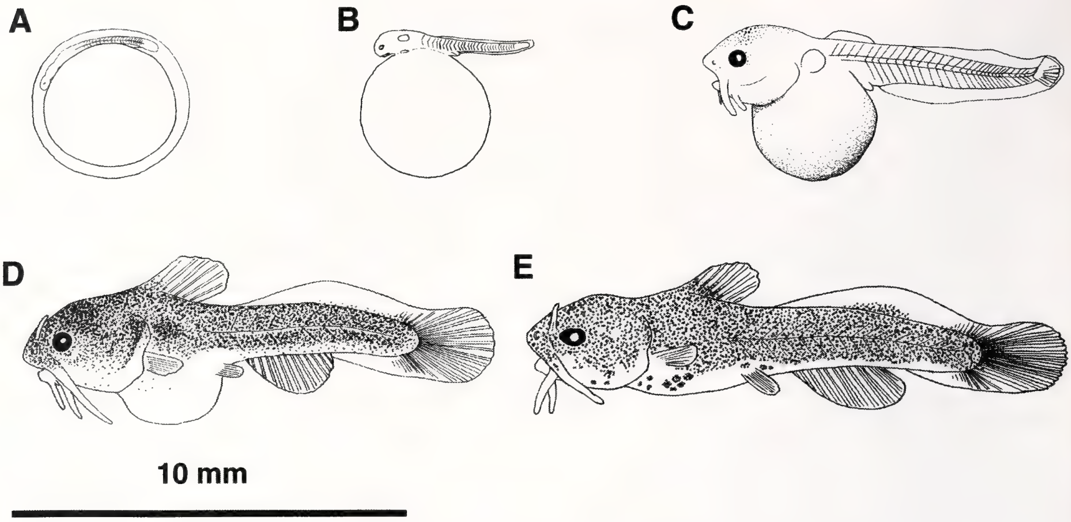

Figure 4. Lateral views of embryos and larvae of *N. stigmosus*. A) 50 hr (postfertilization) embryo, about 3.5 mm TL. B) 96 hr (postfertilization) embryo, 5.2 mm TL, chorion removed. C) hatchling, 8.1 mm TL. D) larva 6 days after hatching, 13.3 mm TL. E) larva 10 days after hatching, 15.4 mm TL.

around tail, and ventrally to forming cloaca; about 28 somites; embryos 5.2 mm TL and wrapped about 45% around yolk sac; strong movements in posterior half of body (Figure 4B).

144 hrs (6 days). Eye with pigment in retina; pigment not present elsewhere on body.

192 hrs (8 days). Mouth well developed; extensive vascularization present along embryo trunk and tail and on yolk sac; pectoral fins prominent; pelvic fins absent.

284 hrs (12 days). First pigmentation appears outside of retina, present as melanophores on dorsum of head.

308 hrs (13 days). All embryos hatched between 284 and 308 hrs. Hatchlings 8.1–9.3 mm TL (n=4). Yolk sac diameter 3.0 mm; about 30 myomeres visible; caudal fin rays forming; other fins without rays; pelvic fin buds present (Figure 4C)

6 days (after hatching). Yolk sac considerably smaller; body well pigmented; a few spots on underside of thoracic region; 7 pectoral rays, 6 pelvic rays, 7 dorsal rays, 13 anal rays, 26 caudal rays. Larvae 13.3 mm TL (n =1) (Figure 4D).

10 days. Young with many spots beneath head and thoracic region; yolk sac completely absorbed in three individuals, nearly absorbed in one individual (Figure 1E); 8–9 pectoral rays, 8–9 pelvic rays, 7–8 dorsal rays, 13 anal rays, 33–34 caudal rays. Larvae 15.4–15.7 mm TL (n = 2) (Figure 4E).

Dispersal of Young

On 21 July 2002, numerous 16–23 mm SL young were collected with a seine (4.8 mm mesh), all confined to the raceway just upstream of the large riffle. By 11 August, young also were found at the top of the riffle. Young *N. stigmosus* collected on 20 September were 28–45 mm SL and were distributed throughout the raceway and riffle complex.

DISCUSSION

Reproductive Biology

Discovered nests, GSI indices, and seasonal variation of external sexual dimorphism all support a relatively short spawning season, from about mid-June to mid-July. *N. stigmosus* reaches sexual maturity at age two and approximately 60 mm SL; females may mature at slightly shorter lengths than males. Some evidence for early maturation (at 13 months) of females was found.

Variation in age at reproductive maturity within populations has been seen in other fish species as a response to predation (Belk 1995), existence of multiple reproductive strategies (Matthews 1998), and ambient environmental

conditions (Bayliss et al. 1993). In *N. stigmosus*, environmental factors are likely to influence age at reproductive maturity. Early maturation in females has been documented in other species of madtoms, including *N. flavipinnis* Taylor (Dinkins and Shute 1996) and *N. flavater* Taylor (Burr and Mayden 1984). This is thought to occur only under optimal conditions of warm winter water and abundant food, based on laboratory maintenance of madtoms (Dinkins and Shute 1996). An unseasonably warm summer with abundant insects could have caused females at Moore's Ferry to grow quickly, potentially reaching sexual maturity near the end of the spawning season, at 13 to 14 months old.

A clutch size of 70–110 is supported by our fecundity studies and nest observations, although small mature females may have smaller clutches (Table 1). This estimate closely follows the relationship between mean fecundity and body size for ictalurids (Burr and Stoeckel 1999), although much variability is seen in potential clutch size among individuals examined in this study.

Comparison of Nesting Biology and Development

The Licking River nests differ from the *N. stigmosus* nests described by Taylor (1969) in the Huron River, Michigan, and by MacInnis (1998) in Lake St. Clair, Ontario, in three respects. First, all Licking River nests were under natural substrates (large slabrocks), while nearly all other reported nests were in refuse (cans, bottles) or prefabricated nest boxes. This may reflect the large amount of potential nest rocks and paucity of cans and bottles at the Licking River site. Second, the Licking River nests contained fewer eggs (55–86) compared to the northern nests (32–141; mean = 97). This is likely due to the larger body size of individuals from northern populations (Taylor 1969; Trautman 1981; MacInnis 1998; Thomas and Burr 2004), or perhaps the largest northern nests may be due to multiple spawning events (MacInnis 1998). Last, guardian males were reported from the northern nests, typical of ictalurids (Burr and Stoeckel 1999), but no guardian males were found with Licking River nests. It seems unlikely that nests were not guarded in the Licking River. There was abundant cover near the nest rocks, especially in the form of dense macrophytic vegetation – perhaps guardian males were hidden nearby.

Embryonic development was slower than reported for other madtoms. Hatching in our study was estimated at about 13 days after fertilization; most other madtoms hatch 6–12 days after fertilization (Burr and Stoeckel 1999). It is likely that development is more rapid in natural conditions, because the lab temperature was cooler (21–22°C) than the Licking River (23–25°C).

Diagnosis of Larvae of *N. stigmosus*

Large melanophores underneath the head and thoracic region distinguish adult *N. stigmosus* from other similar madtoms (Thomas 2002). A few melanophores are present between the pectoral fins of 6-day old larvae. At 10 days, melanophores were prominent between the pectoral fins and a few melanophores were present on the underside of the head. Larvae of other madtoms sympatric with *N. stigmosus* in the Licking River (*N. eleutherus*, *N. flavus*, and *N. miurus*) lack melanophores under the head and between the thoracic fins (Simon and Wallus 2004). In addition, *N. stigmosus* larvae have 13 anal fin rays (15–19 in *N. flavus*), and lack pigment in the distal part of the dorsal fin (present in *N. miurus* by 14 mm TL).

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Fish Species of the Little River Basin, Western Kentucky, 2000–2003

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ABSTRACT

Fish community studies were carried out in the Little River basin of western Kentucky in 2000 and 2003. Thirteen families and 45 species of fish were collected at 16 sites. Thirty-three percent of all taxa were considered intolerant of high turbidity, siltation, and low dissolved oxygen. The most frequently encountered species were scarlet shiner (15 of 16 sites), striped shiner (14 of 16 sites), bluntnose minnow (13 of 16 sites), and longear sunfish at 13 of 16 sites. Other taxa occurring at 75% of all sites included bluegill, largescale stoneroller, banded sculpin, and green sunfish. The highest number of species (21) was found at Site 11 on the Sinking Fork. No federally threatened or endangered species were found. The Little River continues to be an impaired system for aquatic biota.

KEY WORDS: Fish, water quality, streams, agriculture, Little River

INTRODUCTION

In agriculturally impaired rivers and streams, biological homogenization and simplification occur causing the disappearance of unique local fish communities and replacement by "weedy" widespread species more tolerant of human activity (McKinney and Lockwood 2001). Therefore, fish community species composition is an important evaluation of the biotic integrity of impaired stream systems (Karr 1981; Compton et al. 2003).

The Little River, located in the Lower Cumberland River Basin in western Kentucky, drains a variety of land-uses, primarily agricultural, and consequently receives several pollutants from non-point sources. Nutrients, pathogens, and siltation have resulted in serious impairments to the mainstem (KWRRI 1999). Other non-point sources of pollutants include resource extraction, urban-suburban development, urban runoff, and storm sewer discharges proximate to human populations (e.g., Hopkinsville, population ~32,000). Industrial point sources contribute moderate impairments (KWRRI 1999). The Little River is considered a "first priority" river for total maximum daily load (TMDL) development by the Kentucky Division of Water (KDOW 1996; KWRRI 1999).

The purpose of this study was to document fish species composition at 16 sites throughout the Little River Basin during two surveys in 2000 and two surveys in 2003 and to make these data available for future studies. This report complements other biological and physicochemical studies that were part of more indepth multiassemblage biological assessment of the Little River Basin (White et al. 2001; Hendricks et al. 2006a, 2006b; Hendricks and Luttenton 2007).

MATERIALS AND METHODS

Site Selection

The Little River, located in the Lower Cumberland Basin in western Kentucky, is part of the Interior Plateau Ecoregion of Omernik (1987) and also known as the Pennyroyal Ichthyoregion (Compton 2003). Sixteen sites within the Little River and its tributaries were assessed for habitat quality and sampled for fish in 2000 and 2003 (Figure 1). Site locations were based on areas of concern to the Kentucky Division of Water and were selected using USGS maps, GPS data, and GIS information. They included three sites on Sinking Fork Creek, three on Skinner-Casey Creeks, three on the South Fork, four on the

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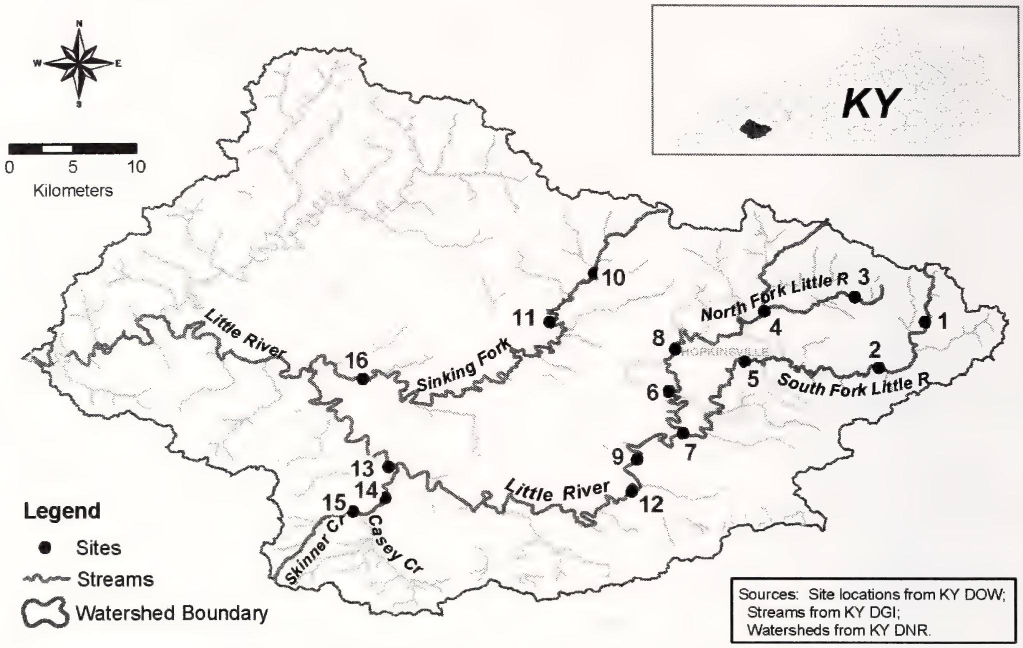

Figure 1. Study reaches in the Little River Basin. Sites 1, 2, and 5 are on the South Fork; Sites 3, 4, 6, and 8 are on the North Fork; Sites 7, 9, and 12 are on the main branch; Sites 10, 11, and 16 are on the Sinking Fork; Sites 15, 14, and 13 are on Casey/Skinner Creeks. Modified from Hendricks and Luttenton (2007).

North Fork, and three on the Little River mainstem. A general description of Little River Basin environmental conditions can be found in Hendricks et al. (2006a).

Fish Collections

In 2000 and 2003, all sites were sampled for fish either in May, June, or July during high base-flow, and again in September or October during low base-flow. In 2000, fish were sampled using a portable backpack electroshocker. In 2003, fish were sampled using both a seine and an electroshocker. In both years, selected 100-meter reaches were intensively shocked and/or seined for approximately 60 minutes at each site. Fish were identified in the field, recorded, and released. Representative fish samples were taken back to the laboratory, fixed in formalin, rinsed with water, and preserved in 40% isopropanol. Specimens were identified and enumerated and are archived at the Hancock Biological Station, Murray, KY.

RESULTS AND DISCUSSION

Fish Species

A total of 13 families and 45 species of fish were collected at 16 sites in the Little River Basin (Table 1), 34 species in 2000 and 42 in 2003. Accepted common names are used in the text, and both common and scientific names are given in Table 1. The most numerous species collected were scarlet shiner (2258 individuals at 15 of 16 sites), striped shiner (569 individuals at 14 of 16 sites), bluntnose minnow (437 individuals at 13 of 16 sites), and longear sunfish (429 individuals at 13 of 16 sites). Other species less numerous but occurring at 75% of all sites included bluegill, largescale stoneroller, banded sculpin, and green sunfish. Species with a single specimen encountered were golden redhorse, blacknose dace, black bullhead, longnose gar, and slenderhead darter. These species are migratory and presumably entered small tributaries within the basin from Lake Barkley in order to breed. The distribution of blacknose dace is sporadic in Kentucky, and it was not reported from the Little River by Burr and Warren (1986). The greatest numbers of species found ($\geq$20) were at Sites 10 and 11 on the Sinking Fork, Sites 6 and 7 downstream from Hopkinsville, and Site 13 near the confluence of Skinner Creek and the mainstem of the Little River.

No federally threatened or endangered species were encountered during either 2000 or 2003 at any of the sites. The smallscale darter is listed as a Kentucky endangered species by the Kentucky State Nature Preserves Commission (2000). It was abundant in the Little River in deep, swift riffles where it was difficult to stand and collect.

A compilation of fish species at several Little River sites from the 1970s and 1980s (Rister 1994) and a KDOW study in 1988 (KDOW 1996) showed results similar to ours. For example, 31 fish taxa were reported in 1988 (KDOW 1996) in the Little River and tributaries. Further, a fairly respectable representation of sensitive *Etheostoma* and *Cottus* sp. was found in the Little River Basin relative to other southeastern agricultural Kentucky streams (Scott 2006). We attribute the higher numbers of species found during this study, in part, to more intensive sampling using both seine and electroshocking methods and to repeated sampling during both years (early and late summer, 2000 and 2003) rather than one summer sampling as had been done in the previous studies.

Thirty-three percent of all fish taxa we found throughout the basin were considered to be intolerant of high turbidity, siltation, or low dissolved oxygen. Most were members of the darter tribe, but they also included northern hog sucker, golden redhorse, longear sunfish, banded sculpin, redtail chub, and rainbow trout. Our data agreed with other studies that found many intolerant species sometimes comprising a significant proportion (e.g., 10–50%) of the fish community at other Little River sites (see Rister 1994).

Our results are roughly comparable with earlier studies from 1988 (KDOW 1996). Recent historical studies (1977–1993) of fish found in the Little River that are intolerant of turbidity, siltation, and low dissolved oxygen included smallmouth bass, longear sunfish, northern hog sucker, spotted sucker, and johnny darter (Rister 1994 and references therein). In Casey Creek, intolerant fish found in 1988 included longear sunfish, northern hog sucker, greenside darter, orangethroat darter, rockbass, rainbow trout (stocked), and banded sculpin. At Sinking Fork in 1993, intolerant fish found included all of the above plus blackside darter and slabrock darter (Rister 1994).

Changes and potential changes in nomenclature are noteworthy since the data were reported. *Erimyzon oblongus* (Mitchill), the creek chubsucker, as originally identified in this study is now *E. claviformis* (Girard), the western creek chubsucker (Bailey et al. 2004). *Rhinichthys atratulus*, the blacknose dace, remains the same (Larry Page, Curator of Fishes, Florida Museum of Natural History, and ASIH/AFS Fishes Names Committee, personal communication).

CONCLUSIONS

Our knowledge of the historical species composition of Little River fish communities is limited. We cannot speculate on long-term fish community trends because we do not know if the intolerant species such as those of the *Etheostoma* and *Cottus* are hanging on due to "land use legacy" areas of the basin that remain less disturbed by human activity (Scott 2006) or if these species were ever abundantly represented basin-wide. Further, species entering the basin to spawn from human-constructed Lake Barkley may temporarily increase the biodiversity of the basin at certain times of the year.

Other habitat assessments carried out in conjunction with this study in 2000 and 2003 (Hendricks et al. 2006a) resulted in "not-supporting" and "partially supporting" classifications for the Little River (assessment data not presented here). Algal assemblages at these sites also indicated degraded aquatic habitats throughout the basin (Hendricks et al. 2006b; Hendricks and Luttenton 2007). We conclude that many parts of the Little River continue to be highly impaired and mostly non-supportive of aquatic biota. Increased human activity in the Little River Basin has contributed to habitat degradation due to the alteration of hydrologic regimes, channel morphology, and water quality including increased siltation, high turbidity, high nutrient loading, and potentially low dissolved oxygen.

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Table 1. Checklist of fish taxa found in the Little River basin by site. Occurrences are noted from 2000 (x/) and 2003(/x) or both (x/x). Sites are ordered from upstream to downstream in respective reaches and correspond to Figure 1.

		South Fork			North Fork				Little River			Sinking Fork			Casey/Skinner			
	Site	1	2	5	3	4	8	6	7	9	12	10	11	16	15	14	13	Total # indiv.
Taxon	Common name																	
Lepisosteidae																		
Lepisosteus osseus (Linnaeus)	Longnose gar																-/x	1
Cyprinidae																		
Campostoma oligolepis Hubbs & Greene	Largescale stoneroller	x/x	x/x	x/x			x/-	x/x	x/x	-/x	-/x	x/x	x/x	x/x	x/x	x/x	x/x	295
Cyprinella spiloptera (Cope)	Spotfin shiner							-/x	-/x	-/x	-/x							12
Cyprinus carpio (Linnaeus)	Carp						x/-	-/x										3
Luxilus chrysocephalus Rafinesque	Striped shiner	x/x	x/x	-/x			-/x	-/x	x/x	-/x	-/x	x/x	x/x	x/x	-/x	x/x	-/x	569
Lythrurus fasciolaris (Gilbert)	Scarlet shiner	-/x	x/x	-/x		-/x	x/x	x/x	x/x	-/x	-/x	x/x	x/x	-/x	x/x	x/x	-/x	2258
Nocomis effusus Lachner & Jenkins	Redtail chub							-/x		-/x	-/x					-/x	-/x	21
Notemigonus crysoleucas (Mitchill)	Golden shiner			x/-										-/x				2
Pimephales notatus (Rafinesque)	Bluntnose minnow	-/x	x/x	x/x	x/x	x/x	x/x	-/x	x/-	-/x		x/x	x/x	-/x			x/x	437
Rhinichthys atratulus (Hermann)	Blacknose dace														-/x			1
Semotilus atromaculatus (Mitchill)	Creek chub	x/x		-/x			-/x					x/x	x/x	x/x	x/x	x/x		112
Catostomidae																		
Catostomus commersoni (Lacépède)	White sucker	x/x	-/x									x/x			x/-			34
Erimyzon claviformis (Girard 1856)	Western creek chubsucker	-/x	x/-		x/-		x/-			-/x		x/x	x/x		x/x			55
Hypentelium nigricans (Leseuer)	Northern hogsucker								x/-	-/x		-/x		-/x			x/x	12
Minytrema melanops (Rafinesque)	Spotted sucker							x/x									-/x	162
Moxostoma erythrurum (Rafinesque)	Golden redhorse															-/x		1
Ictaluridae																		
Ameirus natalis (Rafinesque)	Yellow bullhead	-/x	x/x		-/x	-/x	-/x	x/-				x/x	x/x					50
Ictalurus punctatus (Rafinesque)	Channel catfish						x/-											1
Esocidae																		
Esox americanus vermiculatus Lesueur	Grass pickerel														-/x			2
Salmonidae																		
Oncorhynchus mykiss (Walbaum)	Rainbow trout															-/x		3
Aphredoderidae																		
Aphredoderus sayanus (Gilliams)	Pirate perch					x/-				-/x	-/x		-/x	-/x		-/x		26
Fundulidae																		
Fundulus olivaceus (Storer)	Blackspotted topminnow	x/x	x/x	-/x	x/x	x/x	x/x	x/x	x/-	-/x		x/x	x/x					180
Poeciliidae																		
Gambusia affinis (Baird & Girard)	Mosquitofish	x/-				x/x	x/x	x/x	-/x		-/x	-/x	-/x					85

Table 1. Continued.

		South Fork			North Fork				Little River			Sinking Fork			Casey/Skinner			
	Site	1	2	5	3	4	8	6	7	9	12	10	11	16	15	14	13	Total # indiv.
COTTIDAE																		
Cottus carolinae (Gill)	Banded sculpin		x/x	-/x		-/x		x/x	x/x	-/x	-/x	-/x	x/x	x/x	x/x	x/x	x/x	705
CENTRARCHIDAE																		
Ambloplites rupestrus (Rafinesque)	Rock bass		x/x	x/x		-/x		x/x	x/x	-/x	-/x		-/x			-/x	x/x	94
Lepomis auritus (Linnaeus)	Redbreast sunfish						x/x		x/-									4
L. cyanellus Rafinesque	Green sunfish	-/x	x/x	x/x	x/x	-/x	x/x	x/x	x/x		-/x	x/x	x/x		x/x			134
L. gulosus (Cuvier)	Warmouth				-/x	-/x						x/-	x/-					14
L. macrochirus Raphinesque	Bluegill	x/x	-/x	-/x	x/x	x/x	-/x	-/x	x/x	-/x	-/x	x/x	-/x		x/-	x/x	x/-	132
L. megalotis (Rafinesque)	Longear sunfish	-/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x	-/x		x/x	x/x	x/x			x/-	429
L. microlophus (Günther)	Redear sunfish	-/x														-/x		5
Micropterus punctulatus (Rafinesque)	Spotted bass			-/x				x/-	x/-									4
M. salmoides (Lacèpéde)	Largemouth bass		x/x		x/x	-/x	-/x	x/-				x/x	x/x				-/x	21
PERCIDAE																		
Etheostoma blennioides Rafinesque	Greenside darter			-/x			x/-		x/x	-/x							-/x	9
E. caeruleum Storer	Rainbow darter															-/x	-/x	5
E. crossopterum Braasch & Mayden	Fringed darter	x/x	x/x	-/x	x/-	x/x	x/x	-/x	x/-			x/x	x/x		x/-			213
E. flavum Etnier & Bailey	Saffron darter									-/x				-/x	-/x	x/x	x/x	31
E. microlepidum Raney & Zorach	Smallscale darter								-/x	-/x	-/x						x/x	75
E. simoterum (Cope)	Tennessee snubnose darter	-/x	x/x	x/x	-/x				x/-	-/x			x/x			-/x	-/x	80
E. smithi Page & Vraasch	Slabrock darter					x/x						x/x	-/x					45
E. spectabile (Agassiz)	Orangethroat darter	-/x	-/x	-/x	x/-						-/x			x/-	x/x	x/x	x/-	101
Percina caprodes (Rafinesque)	Logperch									-/x							-/x	2
P. maculata (Girard)	Blackside darrter				-							x/-	-/x					2
P. phoxocephala (Nelson)	Slenderhead darter																-/x	1
SCIAENIDAE																		
Aplodinotus grunniens Rafinesque	Freshwaterdrum			x/x				x/x	x/x									45
																		6457

vision, and database management. Many undergraduate and graduate students, too numerous to name, participated in this study. Additional technical support was provided by Jane Benson of the Mid-America Remote Sensing Center and Carl Woods of the Science Resource Center, Murray State Univeristy. Mike Compton, Greg Pond, John Brumley, and Randall Payne of the Kentucky Division of Water provided advice with methods and analyses. This research was supported by Non-Point Source Implementation Grants (MOA 00151553 and 04103404) from the Department for Environmental Protection/Kentucky Division of Water.

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More New Moth Records (Lepidoptera) from Kentucky

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ABSTRACT

The authors add 41 species in 13 families to the list of known moths (Lepidoptera) of Kentucky. The total known from the state is now 2493 species. The number of species in the families including these new records are Gracillariidae (1), Tineidae (1), Elachistidae (1), Gelechiidae (3), Sesiidae (1), Tortricidae (4), Crambidae (6), Pyralidae (3), Pterophoridae (4), Geometridae (5), Sphingidae (1), Arctiidae (1), and Noctuidae (10).

KEY WORDS: butterflies, moths, Lepidoptera, Kentucky

INTRODUCTION

After 35 years of survey efforts by a substantial number of people, the basic annotated checklist of 2388 species of butterflies and moths of Kentucky in 63 families was published by the Kentucky State Nature Preserves Commission (Covell 1999). A first supplement was published by Covell et al. (2000), adding 35 species to bring the total to 2423. A second supplement (Gibson and Covell 2006) added another 29 making the total 2452 (two additional entries were redundant, having been found to have been published previously). Here we add 41 more species, representing 13 families, to bring the species total to 2493. There are numerous other specimens still to be identified and added to the list at a later date, and members of the Society of Kentucky Lepidopterists and others continue field work to sample new habitats and localities to assist in learning more fully the extent of the lepidopterous fauna of the Commonwealth of Kentucky. In addition, many locality and date entries are being added to the easy-to-use online, interactive database, "Kentucky Butterfly Net" (Marcus et al. 2007). We encourage people to visit www.kybutterfly.net to see information and (for many species) images of our state's lepidopterous fauna. The authors welcome inquiries from those wishing to contribute additional data to the database or who desire assistance in identification of unknown specimens and images.

Specimens on which the present new records are based are housed in collections of the University of Kentucky Department of Entomology, The McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, FL, and the persons indicated as collectors of the various species. Determinations not attributed to individuals in each entry were made by the collector and confirmed by the authors.

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ADDITIONS TO SPECIES LIST:

Numbers refer to Hodges et al. (1983) check list.

Family TINEIDAE

307.1 *Dryadaula* undescribed species.

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Menifee County, Road 9B, Indian East Fork, Kelley Branch, elevation 720 feet, 21 July 1985, John S. Nordin. Determined by Don R. Davis.

Family GRACILLARIIDAE

622 *Caloptilia paradoxa* (Frey and Boll).

Jefferson County, 27 March 1981, David Flaim. Determined by Jean-Francois Landry.

Family ELACHISTIDAE

1124 *Biselachista cucullata* (Braun).

Wolfe County, Silvermine Arch near Koomer Ridge Campground, 10 June 1976, collected and determined by John B. Heppner.

Family GELECHIIDAE

1719 *Chrysoesthia sexguttella* (Thunberg)

Louisville, Jefferson County, 2 June, 1982, in mosquito survey light trap. Determined by Jean-Francois Landry.

1789 *Coleotechnites apicitripunctella* (Clemens).

Wolfe County, Silvermine Arch near Koomer Ridge Campground, 10 June 1976, collected and determined by John B. Heppner.

2294.1 *Dichomeris gleba* Hodges.

Larue County, in prairie habitat, 30 May 2006, Loran D. Gibson.

Family SESIIDAE

2567 *Synanthedon rubrofascia* (Henry Edwards).

Whitley County, Butcher Hollow near Woodbine, 21 June 2001, Leroy C. Koehn.

Family TORTRICIDAE

2863 *Hedya chionosema* (Zeller).

Rowan County, Morehead, 15 June 2006, Jonathan Smith, at lights; Owsley County, near Booneville, 6 June 2007, L. D. Gibson in light trap.

2940 *Phaneta convergana* (McDunnough).

Larue County, prairie habitat, 3 May 2006, Loran D. Gibson. Determined by Donald J. Wright.

3397 *Hystrichophora loricana* (Grote).

Known until a few years ago only from the holotype from Ohio, this rare species was discovered in Lincoln County, near Stanford, 28 August 2008 by Loran D. Gibson.

3731 *Sparganothis lentiginosana* (Walsingham).

Bell County, Pine Mountain State Park, 9 June 1981, Carl C. Cornett in blacklight trap. Determined by John W. Brown.

Family CRAMBIDAE

5037 *Pyrausta inornatalis* (Fernald).

Lexington, Fayette Co., seen and photographed 21 January 2008, by Cynthia L. Higgs in her kitchen. She photographed and collected a second specimen on 21 July 2008 outside her home. Images, information and the second specimen were transmitted to Dr. Ken Yeargan, University of Kentucky, who relayed it to Covell. Another individual was photographed by Frank Lyne in Logan County near Dot, 8 July 2008, identified by David Roemer. It was previously known from Florida, Texas, Oklahoma, Mississippi, and Tennessee, and associated with *Salvia*, which may be its foodplant (information from http://www.davesgarden.com).

5189 *Blepharomastix rehamalis* (Dyar).

Larue County, prairie habitat, 30 May and 31 July 2006, Loran D. Gibson; Hart County, near Bonnieville, 27 May 2006, Richard Healy; Meade County, Lapland Road near Battletown, 18 July 2005, Loran D. Gibson.

5215 *Condylorrhiza vestigialis* (Guenée).

Owsley Co., near Booneville, 30 September 2006, Loran D. Gibson. Determined by James T. Vargo.

5299 *Acentria ephemerella* (Denis and Schiffermuller). Water Veneer Moth; Watermilfoil Moth.

McCracken County, Mayfield Creek bottoms near Lone Oak, 6 October 1989, Charles V. Covell Jr. at light. Determined by Jean-Francois Landry. This species was listed in Hodges et al. (1983) as *Acentria nivea* (Olivier).

5366 *Crambus watsonellus* Klots.

McCracken County, Paducah, "zip track site," 9 September 2006, Loran D. Gibson. Determined by George J. Balogh.

5392 *Arequipa turbatella* Walker.

Menifee County, Daniel Boone National Forest, Red River Gorge, Leatherwood Fork, Forest Road 9A, 12 July 2007, Loran D. Gibson.

Family PYRALIDAE

5625 *Omphalocera cariosa* Lederer.

Hart County, near Bonnieville, 13 July 2006, Richard Healy.

6043 *Peoria bipartitella* Ragonot.

Larue County, in prairie habitat, 30 June 2006, Loran D. Gibson.

6049 *Peoria roseotinctella* (Ragonot).

Larue County, in prairie habitat, 31 July 2006, Loran D. Gibson.

Family PTEROPHORIDAE

6120 *Lioptilodes albistriolatus* (Zeller).

Hart County near Bonnieville, 7 June 2006, 3 September 2007, 23 September 2004, 28 September 2005, 28 October 2005, 30 October 2007, and 1 November 2007; Richard Healy. Determined by Debbie M. Lott.

6183 *Hellinsia citrites* (Meyrick).

Hart County near Bonnieville, 27 June 2007, Richard Healy. Determined by Debbie M. Lott.

6212.1 *Hellinsia chlorias* (Meyrick).

Hart County near Bonnieville, 30 July and 28 August 200?, Richard Healy. Determined by Debbie M. Lott.

6226 *Hellinsia unicolor* (Barnes and McDunnough).

Hart County near Bonnieville, 25 July 2007, Richard Healy. Determined by Debbie M. Lott.

Family GEOMETRIDAE

6780 *Ceratonyx satanaria* Guenée.

Hart County, near Bonnieville, 20 and 25 March 2005, 1 April 2006, and 20 March 2007, Richard Healy. Determined by Charles V. Covell Jr. A significant northward extension of the known range of this species.

6833 *Metarranthis mollicularia* (Zeller).

Ballard County, Boatright Wildlife Management Area, Prairie Lake Field, 12 August 2007, Gerald D. Burnett. Determined by James K. Adams. Another significant northward range extension.

6936 *Eusarca packardaria* (McDunnough).

Ballard County, Ballard Wildlife Management Area, Scenic Trail, 11 September 2008, Gerald D. Burnett. Determined by James K. Adams.

7437 *Operophtera bruceata* (Hulst).

Oldham County, Old Taylor Place, Goshen, 23 November 1993, 25 November 1995, 1 December 1995 and 14 December 1995, Robert V. Gregg. Determined by C. V. Covell Jr.

7640 *Lobophora nivigerata* Walker.

Rowan County, Morehead, 11 May 2001, Jonathan Smith. Determined by Charles V. Covell Jr.

Family SPHINGIDAE

7860 *Eumorpha intermedia* (B. P. Clark).

Carlisle County, Doug Travis Wildlife Management Area (formerly Wesvaco Wildlife Management Area), 12 and 25 June 2004, 18 June 2005, Gerald Burnett. Determined by James K. Adams. A significant northward range extension.

Family ARCTIIDAE

8217 *Leucanopsis longa* (Grote).

Calloway Co., north side of Grubbs Road, 15 miles southeast of Murray, 16 June 2007, Loran D. Gibson.

Family NOCTUIDAE

8431 *Schrankia macula* (Druce).

Massac Creek bottoms, Paducah, McCracken Co., 5 October 1999, in UV trap near cane break, James T. Vargo; Ballard County, Ballard Wildlife Management Area, Old Hunter Check Station, 19 October 2004, and Little Turner Lake, 20 October 2004, collected and determined by Gerald D. Burnett.

9005 *Tripudia balteata* Smith.

McCracken County, Paducah, 955 Smith Ave., G. D. Burnett. Determined by James K. Adams.

9098 *Tarachidia phecolisca* (Druce).

Carlisle County, Doug Travis Wildlife Management Area, 20 August 2004, Gerald D. Burnett. Determined by James K. Adams.

9426 *Meropleon titan* Todd.

Ballard County, Blandville Cemetery, 3 September 2006, Gerald Burnett. Determined by James K. Adams.

9498 *Papaipema silphii* Bird.

Rowan County, Clack Mountain, Rt. 1274, 2 and 22 October, 2007, Jonathan Smith. Determined by Loran D. Gibson.

9670 *Spodoptera latifascia* (Walker).

Ballard County, 233 Gray Road, 6 October 2007, Gerald Burnett. Determined by James K. Adams.

10628 *Tricholita notata* Strecker.

McCracken County, Paducah, "zip track site," 22 September 2007, Gerald Burnett.

10694 *Eucoptocnemis fimbriaris* (Guenée).

Ballard County, Ballard Wildlife Management Area, deer barn, 10 October 2006, Gerald D. Burnett. Determined by James K. Adams.

11012.1 *Noctua pronuba* (Linnaeus). Large Yellow Underwing.

Our earliest record of this recent introduction from Europe to North America is: Rowan County, Morehead, 13 August 2000, Jonathan Smith. The moth is statewide and common now. Here are a few additional records: Harlan County, top of Big Black Mountain, 4100 feet elevation, July 6, 2002, C.V. Covell Jr.; Hardin County, 12 miles SW of Elizabethtown, 15 August 2003; Rowan County, Rt. 1274, 5 miles south of Morehead, 8 July 2005; Larue County, 4 miles west of New Haven, 14 August 2006 (these last three collected by L. D. Gibson).

11055 *Derrima stellata* Walker. Pink Star Moth.

Hart County, near Bonnieville, 8 and 17 July 2007, and 20 July 2008; Richard Healy.

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NOTES

Experimental Infections of Bluegill, *Lepomis macrochirus* Rafinesque, with Cercariae of the Digenean, *Proterometra macrostoma* (Faust): (I) Infectivity of the Embryonic Cercaria and (II) Initiation of Egg Development—The mature redia of the digenean, *Proterometra macrostoma* (Faust), contains a single, "embryonic" progenetic (i.e., developed sexual organs) cercaria and several smaller cercariae that are less differentiated (Horsfall 1934; Rosen et al. 2005a) (Figure 1a). Just prior to *P. macrostoma* emergence from its snail intermediate host, the distome body of the largest cercaria in the redia completely withdraws into its cercarial tail (Figure 1b). The withdrawl process likely serves as a mechanism for streamlining the cercaria so that it can engage in its distinctive swimming behavior in the water column that serves to attract the fish definitive host (Prior and Uglem 1979). In this regard, Horsfall (1934) noted that the drag produced by the distome attached to the anterior end of the cercarial tail (and not withdrawn) prevented effective swimming in this species. In addition, the withdrawl of the distome body protects it from osmotic stress encountered by the worm following its release from the snail into a hypotonic, freshwater environment (Braham et al. 1996; Braham and Uglem 2000). While it is clear that distome withdrawl sustains maximal infectivity during the cercaria's 14–20 hr swimming phase (Braham et al. 1996), it is not known whether or not the distome body is infective to the fish definitive host prior to this retraction.

It also is well known that the progenetic cercaria of *P. macrostoma* may or may not contain eggs in early stages of development (Dickerman 1945; Rosen et al. 2005b) (Figure 1c). However, in those cercariae lacking eggs, it is not clear what time frame is required for the initiation of egg production once the cercaria is ingested by an appropriate centrarchid fish definitive host. The following study was initiated to address these two questions.

Bluegill, *Lepomis macrochirus* Rafinesque, were obtained from the Pfeiffer Fish Hatchery near Frankfort, Kentucky, held at 22.7 ± 4.6°C, and maintained on fish pellets obtained from the hatchery. Three fish were placed in smaller, 1-gal tanks for exposure to cercariae. Snails, *Elimia semicarinata* (Say), previously screened for patent (i.e., shedding cercariae) *P. macrostoma* infections as described by Rosen et al. (2000), were placed into enamel pans, crushed, and 3–5 of the largest embryonic (i.e., body not withdrawn into the tail) cercariae were pipetted into the tanks with individual fish. As embryonic cercariae are poor swimmers, a 10-ml pipette was used when necessary to keep worms suspended in the water column to attract fish until the former were ingested. At 2 and 5 days PI (postinfection), bluegill were anesthetized in MS-222 (tricaine methane sulfonate), sacrificed, and necropsied. The number of worms recovered along with the number of eggs within these worms were recorded. For the second experiment, freshly emerged cercariae

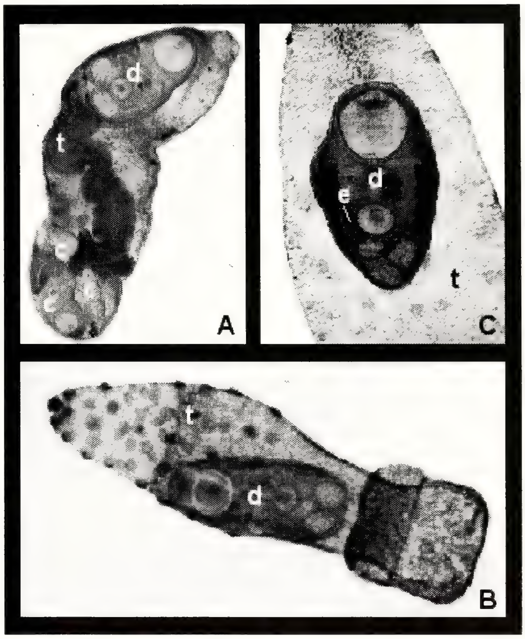

Figure 1. Intramolluscan stages of the digenetic trematode, *Proterometra macrostoma*: (A) Mature redia with embryonic cercariae; 40× (B) Mature cercaria with distome body retracted into tail; 40× (C) Mature cercaria with eggs in distome; 100×; c = younger embryonic cercariae, d = distome body, e = young eggs, t = cercarial tail.

were examined with a compound microscope to determine the presence or absence of eggs. Seventeen young bluegill were then individually placed into one gallon tanks and exposed to 1–4 four cercariae with (8 fish) or without (9 fish) eggs. Once all introduced cercariae were ingested, the fish were returned to their respective 10-gal holding tanks. On day 1, PI, fish were anesthetized as previously described for recovery of worms and the number of eggs in these worms recorded.

Developing adult worms were recovered from all 3 fish exposed to embryonic cercariae with bodies not retracted into their tails. Day 5 PI worms (N = 6) recovered from 2 fish had an average of 51.5 ± 13.2 eggs indicating the progression of normal development in the fish definitive host. Sixteen worms were recovered from fish exposed to cercariae lacking eggs and 15 worms from fish exposed to cercariae with eggs on day 1 PI. Adult worms derived from the former had an average of 13.9 ± 1.3 eggs, while adult worms obtained from cercariae with eggs had an average of 21.7 ± 2.3. These averages were significantly different when assessed with a Student's *t*-test ($t = 2.868$; df = 29; $P = 0.008$).

In summary, the distome body of the *P. macrostoma* cercaria was infective to bluegill prior to the retraction of

the body into its tail. However, distome withdrawl into the tail, though apparently not promoting changes in distome physiology related to infectivity as shown in this study, is an essential process for the completion of this parasite's life cycle. The tail serves as a protective "vehicle" for increasing the probability that the "infective" distome will be ingested by a proper fish definitive host. Within 1 day following ingestion by a bluegill definitive host, *P. macrostoma* adults derived from cercariae lacking eggs showed significant egg production, although the average number of eggs was significantly less than that observed in adult worms obtained from cercariae with eggs. Thus, the initiation of egg production is rapid (i.e., within 24 hr) once the worm is exposed to "triggers" in the esophagus or stomach of the fish definitive host.

This study was supported by a grant from the Undergraduate Research and Creative Projects Program (URCPP) at Berea College to Ronald Rosen. We acknowledge Steve Marple and Nick Scudlarek for providing us with young bluegill from the Pfeiffer Fish Hatchery near Frankfort, KY.

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Abstracts of Some Papers Presented at the 2008 Annual Meeting of the Kentucky Academy of Science

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AGRICULTURAL SCIENCES

Yield and Quality of Vegetables Grown in Sewage Sludge Amended Soil. REGINA R. HILL*, ERIC TURLEY and GEORGE F. ANTONIOUS, Land Grant Program, Department of Plant and Soil Science, Kentucky State University, Frankfort, KY 40601.

Sewage sludge as a soil amendment is a simple and inexpensive way to improve soil fertility and physical properties of agricultural soils. The nutrients in biosolids can replace commercial fertilizer, while the biosolids organic matter can improve crop yield and quality. The objective of this study was to compare yield and quality of squash, eggplant, bell pepper, and tomato grown under three soil management practices. The soil management practices were: 1) municipal sewage sludge, 2) municipal sewage sludge mixed with yard waste compost, and 3) rototilled bare soil used for comparison purposes.

Six replicates of each soil treatment were established in 18 plots of 22 × 3.7 m each at Kentucky State University Research Farm, Franklin County, KY. The use of sludge in land farming must increase profits in order for it to become an accepted practice among vegetable growers. Mature fruits were harvested from each plot, weighed and graded according to USDA standards. Yields of squash (3,302 lb/acre), eggplant fruits (5,559 lb/acre), and bell pepper fruits (2,008 lb/acre) obtained from sludge mixed with yard waste compost at 15 tons/acre were superior compared to other treatments. Tomato marketable yield from sludge-yard waste and sludge treatments were not significantly different. However, tomatoes grown under both sludge treatments had significantly higher yields than those grown in no mulch treatments.

A Simplified Procedure for Glucosinolates Quantification. GEORGE ANTONIOUS* and MICHAEL BOMFORD, Community Research Service, Department of Plant and Soil Science, Kentucky State University, Frankfort, KY 40601 and PAUL VINCELLI, Department of Plant Pathology, University of Kentucky, Lexington KY 40546.

Glucosinolates (GSLs), secondary metabolites of *Brassica* plants, are precursors of many natural pesticides, including volatile biofumigants. Consistent and reliable soil-borne pest management with *Brassica* GSLs requires simple, accurate, and fast methods of GSL separation and quantification. The objectives of this investigation were: 1) to develop a simplified procedure for quantification of GSL in *Brassica* accessions and 2) to determine variation in total GSL and phenol concentrations between plants grown under greenhouse, high tunnels and field conditions. A survey of *Brassica* accessions from the national germplasm repository was conducted to identify potential cover crops that could be soil-incorporated for use as biofumigants. Separation of GSLs from the selected *Brassica* accessions was achieved using ion-exchange sephadex in disposable pipette tips. Quantification of total GSLs was based on liberation of the glucose moiety from the GSL molecule by addition of standardized thioglucosidase (myrosinase) and colorimetry. GSL concentration of greenhouse, high tunnel, and field-grown shoots (leaves and stems) averaged 23, 40 and 76 μmoles/g fresh weight, respectively. A comparison of accessions revealed that Ames 8887 of *B. juncea* contained the greatest GSL concentration, but had the lowest biomass yield and ascorbic acid concentration, in part because phytochemical concentration tended to be negatively correlated with biomass yield. More promising was *B. juncea* accession 'Pacific gold' which coupled high biomass yield with above-average GSL production, but had low phenol and ascorbic acid concentration. We conclude that environmental stress on growing plants can increase the concentration of GSLs and total phenols in *Brassica* plants, but does not increase yields of active phytochemicals per unit area.

Ascorbic Acid and Phenol Contents of Hot Pepper Fruits from Eight Countries of Origin. LAUREN LOBEL* and GEORGE F. ANTONIOUS, Land Grant Program, Department of Plant and Soil Science and TEJINDER S. KOCHHAR, Department of Biology, Kentucky State University, Frankfort, KY 40601.

Capsicum chinense has been referred to as the most cultivated pepper in South America. The USDA pepper (*Capsicum* spp.) germplasm collection contains several thousand members or accessions. Many of these species and cultivars have not been analyzed for their concentrations of antioxidant compounds. The main objective of this investigation was to select candidate accessions of hot pepper having high concentrations of ascorbic acid and phenolic content among countries of hot pepper origin for use as parents in breeding for these compounds. Seeds of 63 hot pepper accessions of *C. chinense* were collected from Belize, Brazil, Colombia, Ecuador, Mexico, Peru, Puerto Rico, and United States. Seeds were field grown in a silty-loam soil and their mature fruits were analyzed for ascorbic acid and phenol content. Fruits of *C. chinense* PI-152452 (Brazil) and PI-360726 (Ecuador) contained

* indicates presenter

the greatest concentrations of ascorbic acid (1.2 and 1.1 mg/g fresh fruit, respectively), while PI-438648 (Mexico) contained the greatest concentration of total phenol content (349 µg/g fresh fruit) among the 63 accessions tested. These accessions were identified as potential candidates for mass production of major antioxidants that have health-promoting properties.

Absorption and Accumulation of Heavy Metals in Vegetables Grown in Soil-Mixed with Sewage Sludge. MALEKA EMBRY* and GEORGE ANTONIOUS, Land Grant Program, Department of Plant and Soil Science, and TEJINDER KOCHHAR, Department of Biology, Kentucky State University, Frankfort, KY 40601.

The use of municipal sewage sludge (MSS) as a source of nutrients in crop production is increasing in the U.S. and worldwide. Recycling this material as a soil amendment would reduce the need for landfill disposal and/or incineration and the impact of disposal on environmental quality. Field studies were conducted at the Kentucky State University Research Farm to determine the concentration of seven heavy metals (Cd, Cr, Ni, Pb, Zn, Cu, and Mo) in sewage sludge and yard waste compost, and monitor heavy metal concentration in edible portions of plants at harvest. In consecutive years the soil was incorporated with MSS and planted with potato, sweet pepper, broccoli, squash, and eggplant. Quantitative analyses of extractable metals using inductively coupled plasma (ICP) showed that Cd, Cr, Ni, and Pb in potato tubers, sweet peppers, and broccoli grown in sludge-amended soil were not significantly different from control plants. Concentrations of Zn, Cu, and Mo were significantly greater in potato tubers and sweet peppers grown in sludge compared to their respective controls. Zinc and Mo in broccoli heads were higher than their control plants. Soil analysis during the five years of the study revealed that Zn and Cu have increased significantly in soil as a result of sludge addition. Plant uptake is one of the main pathways through which metals enter a food chain. The impact of potentially toxic trace metals in sludge applied to cropland can be reduced by growing crops that do not accumulate these metals in their edible portions.

Heavy Metal Concentrations in the Fruits of *Capsicum chinense*. GEORGE F. ANTONIOUS, Land Grant Program, Department of Plant and Soil Science, Kentucky State University, Frankfort, KY 40601.

Presently, one of the pollutants of most concern around the world is heavy metals. Elevated concentrations of heavy metals in edible plants could expose consumers to excessive levels of potentially hazardous chemicals. Plant uptake is one of the main pathways through which metals enter the food chain. The main objectives of this investigation were: 1) to select candidate accessions of hot pepper having high concentrations of capsaicin and dihydrocapsaicin for use as parents in breeding for these two compounds, 2) to determine the concentrations of seven heavy metals (Cd, Cr, Ni, Pb, Zn, Cu, and Mo) in soil and their accumulation in hot pepper fruits, and 3) to determine if the heavy metal content of hot pepper fruit that have elevated levels of capsaicin and didydrocapsaicin are lower than the permitted heavy metal limits. Twenty-three genotypes of hot pepper seeds from the USDA germplasm collection were grown in the field to identify accessions having increased concentrations of heavy metals in mature fruits. Concentrations and relative proportions of capsaicin, dihydrocapsaicin, and seven heavy metals varied between and within pepper species. Plant Introduction 547069 (*C. annuum*) contained the greatest concentrations of the two pungent compounds. Fruits of PI-439381 and PI-267729 (*C. baccatum*) accumulated the greatest concentrations of Pb, while PI-246331 (*C. annuum*) accumulated the greatest concentration of Cd among accessions tested.

Conifer Decline and Potential Causes at Baker Arboretum. RICK HEAVRIN* and MARTIN STONE, Western Kentucky University, Department of Agriculture, Bowling Green, KY 42101.

The Baker Arboretum is a fifteen-acre private garden established over fifteen years ago near Bowling Green and serves as a horticultural teaching and research facility for students and faculty at Western Kentucky University. The horticultural collections specialize in dwarf conifers, Asian maples, and American and Asian dogwoods, and their hybrids. Over the past few years, an apparently non-pathogenic decline has been seen in the growth of some conifers. By noting the position of the terminal bud scar, textural, and morphological changes in the stem, the growth of thirty-three specimens from four genera has been tracked over the past four years. Growth of thirteen taxa was significantly reduced for the years 2006 and 2007, compared to the previous years' growth (2004 and 2005). Growth of all taxa combined revealed that in 2008 plants grew more than in 2007. Taxa showing improved growth included cultivars of *Cedrus atlantica* and *Chamaecyparis obtusa*. Taxa not growing significantly more than the previous season's low were species and/or cultivars of *Chamaecyparis pisifera*, *Chamaecyparis gracilis*, *Picea orientalis*, *Picea pungens*, and *Pinus nigra*. There were no taxa showing negative growth compared with the previous year. Tests revealed soil pH between 7.0 and 7.6, which was elevated for these acid-loving plants. Base saturation for soil calcium was excessive compared to magnesium. Irrigation water did not contain excessive calcium but was low in magnesium, which may be a contributing factor.

Investigation of Yield and Quality of Grafted, Heirloom, and Hybrid Tomatoes. STEPHEN FLOMO*, DIANA EDLIN, MARTIN STONE and ELMER GRAY, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101 and NATHAN HOWELL, University of Kentucky Extension, Munfordville, KY 42765.

Tomatoes (*Lycopersicon esculentum* Mill.) are one of the most popular vegetable crops grown for fresh market

and processing in the U.S. Grafting has potential in the U.S. for managing soil-borne diseases, especially for organic heirloom tomato production. The objectives of this study were to examine the effects of grafting heirloom and hybrid cultivars reciprocally and also to determine the impact of self grafting on tomato fruit size, weight, number, and quality. Our cultivars included the heirlooms 'Cherokee Purple' and 'Mister Stripey', and the commercial standards, 'Crista' and 'Maxifort'. The trial was conducted at the WKU Farm in Bowling Green, KY. Transplants were set out on raised beds utilizing drip irrigation to the amount of one acre-inch water each week. The largest fruits were produced by 'Cherokee Purple' due its genetic potential. Fruit size was greatest when 'Cherokee Purple' scion was grafted onto 'Maxifort' rootstock. 'Crista', the hybrid, produced the highest quality fruits regardless of the rootstock. 'Mister Stripey' was more prolific in fruit number but their quality was lower. There was no advantage to self-grafting. One-way analysis of variance using ANOVA with $P<0.05$ was used to determine differences among treatments. Means were separated using the Duncan's multiple range test.

Heirloom and Hybrid Tomato Yield in Organic and Conventional Production Systems. DIANA EDLIN*, MARTIN STONE, STEPHEN FLOMO and ELMER GRAY, Western Kentucky University, Department of Agriculture, Bowling Green, KY 42101 and NATHAN HOWELL, University of Kentucky Extension, Munfordville, KY 42765.

Tomatoes are one of the most valuable vegetable crops grown in the U.S. In 2007, 120,000 acres of tomatoes were grown for fresh market production at a value of 1.3 billion dollars. Of this amount, 578 acres were grown in Kentucky. There is much potential in Kentucky for further expanding production, especially in the niche markets such as heirlooms and organic production. We compared yield of two heirloom cultivars, 'Mr. Stripey' (MS) and 'Cherokee Purple' (CP), to a commercial standard, 'Crista' (CR), under two production systems, organic and conventional. Plants were grown on plastic mulch, in raised beds, with drip irrigation beneath plastic, and received one acre-inch of water per week. This was part of a larger study investigating the influence of grafting heirlooms onto commercial rootstocks and their reciprocal grafts. Planting occurred in late May while harvest began August 1st and continued two and three times a week through the end of the season. Organically grown CP yielded significantly heavier and larger fruits compared to those grown conventionally. CR yielded heavier and larger fruits when grown conventionally. MS also yielded the largest fruits when grown conventionally compared to organic. There were no significant differences between cultivars for number of fruit per plant. All cultivars produced higher fruit quality when grown conventionally. For the organically produced heirloom market, CP is the preferred cultivar.

Impact of Red and Black Plastic Mulch on Yield of Field Grown, Staked Tomatoes. BRANDON BURCHETT*, STEPHEN FLOMO, DIANA EDLIN, ELMER GRAY and MARTIN STONE, Western Kentucky University, Bowling Green, KY 42101 and NATHAN HOWELL, University of Kentucky Cooperative Extension, Munfordville, KY 42765.

Staked, field-grown fresh tomatoes hold much opportunity for Kentucky's agricultural entrepreneurs. The opportunity is especially great for the niche fresh market heirloom cultivars. Colored plastic mulch has been shown to impact crop yields but its effect on Kentucky's tomatoes was unknown. We compared the heirloom cultivars, 'Mister Stripey' and 'Cherokee Purple' to the commercial standard, 'Crista'. This was part of a larger study in which the aforementioned cultivars and the commercial rootstock 'Maxifort' were reciprocally grafted and all were grown conventionally or organically. Tomatoes were planted in the field in late May at the Western Kentucky University farm in Bowling Green, KY. Plants were grown on raised beds with drip irrigation under the plastic under red or black plastic mulch. Beginning in early August, harvests were conducted two or three times per week. Fruits grown on red mulch were significantly ($P<0.05$) heavier and of better quality than those grown on black plastic. However, plants grown on black plastic mulch yielded more but smaller fruits.

Evaluation of Baby Corn as a Niche Crop for Kentucky. TARA HOLADAY* and CRYSTAL WALKER, Transylvania University, Lexington, KY, 40508 and MARTIN STONE, ELMER GRAY and TODD WILLIAN, Western Kentucky University, Department of Agriculture, Bowling Green, KY 42101.

Baby corn is the edible, young, unfertilized ears of corn and is usually consumed fresh or canned. A field trial was conducted during the 2008 growing season at the Western Kentucky University research farm. Three cultivars of field corn were investigated, each with varying degrees of genetic flex. Plots were planted on 30 inch rows, four rows wide, thirty feet long, and data was taken on all rows except the outermost. Three plant population densities of each flex cultivar were planted at 36,000, 46,000, and 57,000 plants per acre. Baby corn was hand harvested on two to three day intervals beginning at 52 DAP (days after planting) and continued for eight harvests through 69 DAP. The highest planting density produced the greatest weight and number of ears and also improved the marketability of ears. The high flex trait produced the greatest weight and number of ears and also increased their marketability. We conclude that producers should plant high flex corn varieties at high densities for optimum baby corn production with the least amount of unmarketable ears.

Populations of Lady Beetles and Lacewings in Organically Grown Sweet Corn Using PredaLure® Insect Attractant. JOHN D. SEDLACEK*, KAREN L. FRI-

LEY, LESLYE S. BRENT and MICHAEL K. BOMFORD, Community Research Service, Kentucky State University, Frankfort, KY 40601.

Sweet corn, *Zea mays* 'Garrison', was grown in 260 m^2 replicated plots using organic production practices. Plots were treated with PredaLure® or were left as untreated controls. One lure was fastened to a tobacco stick placed in the center of the plot and in the center of each plot quadrant. Beneficial insects were sampled weekly during silking using 232 cm^2 yellow sticky traps stapled to each tobacco stick 2.5 cm below each lure. Pink lady beetles, *Coleomegilla maculata*; Asian lady beetles, *Harmonia axyridis*; spotless lady beetle, *Cycloneda munda*; seven-spotted lady beetle, *Coccinella septempunctata*; parenthesis lady beetle, *Hippodamia parenthesis*; convergent lady beetle, *Hippodamia convergens*; big eyed bug, *Geocoris punctipes*, and green lacewing, *Chrysoperla carnea*, were the predatory insects collected on the traps. Pink lady beetle was the most abundant predator caught followed by the big eyed bug. All other predators were not abundant. There was a tendency toward higher numbers of multicolored Asian lady beetles, spotless lady beetles and green lacewings in plots where PredaLure lures had been deployed. However, there were no significant differences in abundance of any of the predatory insects found between PredaLure baited and non baited plots in organically grown sweet corn. This could be due to baited and non baited plots being too close to one another and methyl salicylate plumes saturating both. Other possible explanations may involve the rate of emission of the methyl salicylate from the dispenser or suboptimal placement of the lures in the crop.

Impact of Ripe Pawpaw Fruit Extract on Mortality and Feeding Deterrence of Striped Cucumber Beetles on Squash. KAREN L. FRILEY*, JOHN D. SEDLACEK, JEREMIAH D. LOWE and KIRK W. POMPER, Community Research Service, Land Grant Program, Kentucky State University, Frankfort, KY 40601.

Laboratory experiments were performed to study the effects of pawpaw (*Asimina triloba*) fruit extract on mortality and feeding deterrence of striped cucumber beetle (*Acalymma vittatum*). Acetogenins were extracted from ripe pawpaw fruit pulp using ethyl alcohol. Concentrations of 0, 10, 100, 1,000, 10,000 and 50,000 ppm were used. Buttercup squash leaf disks 3.5 cm in diameter were treated individually with each concentration and placed on water moistened filter paper in 9 cm plastic Petri dishes. Five striped cucumber beetles were placed on each treated or control leaf disk. Feeding activity was recorded in each Petri dish one and four hours after beetle introduction. All Petri dishes were then placed in an environmental growth chamber set at 27°C and a 16:8 hr light:dark photoperiod. After 24 hr the cucumber beetles were removed. Amount of leaf tissue eaten was calculated by tracing the damaged leaf disks using graph paper and a light table. Beetles did not feed on treated squash leaves at either one or four hours of exposure. At 24 h striped cucumber beetle mortality was 35%, feeding was lowest and feeding damage least (<1%) on 50,000 ppm pawpaw treated leaf disks. Additional experiments need to be conducted to determine the optimal concentration of ripe pawpaw fruit extract for striped cucumber beetle feeding deterrence. The duration of treatment effectiveness and susceptibility of other pest and beneficial insect pest species to the extracts also need to be determined.

Populations of Lady Beetles and Green Lacewings in Organic, Conventional and Genetically Engineered (Bt) Sweet Corn. LESLYE S. BRENT*, KAREN L. FRILEY and JOHN D. SEDLACEK, Community Research Service, Kentucky State University, Frankfort, KY 40601.

Lady beetles (Coleoptera: Coccinelidae) and green lacewings (Neuroptera: Chrysopidae) are predators of many small and soft bodied insect pests in agroecosystems. Economically important insects that they prey upon in sweet corn fields include eggs and small larvae of corn earworm, *Helicoverpa zea*; European corn borer, *Ostrinia nubilalis* (Hübner); southwestern corn borer, *Diatreae grandiosella*; and fall armyworm, *Spodoptera frugiperda*. Sweet corn was grown using organic, conventional, and genetically engineered (Bt) production practices. Concerns regarding negative impacts on biodiversity and non target beneficial insects in genetically engineered crops and those which have been treated with broad spectrum insecticides have been voiced. Therefore, the objective of this research was to determine lady beetle species composition and abundance and green lacewing abundance in the three cropping methods of sweet corn. Yellow sticky traps 232 cm^2 in area were used to capture flying insects at tassel and silk height during anthesis in 2006 and 2007. Eight sticky traps were placed equidistant from edges and from each other within the middle row of the center corn subplot in each plot. Sticky traps were serviced weekly for 3 weeks. Pink or 12 spotted lady beetle, *Coleomegilla maculata*, was the most abundant lady beetle caught. Asian multicolored lady beetles, *Harmonia axyridis*, along with six other species of lady beetles, and green lacewings, *Chrysoperla carnea*, were captured but were not abundant. Warrior® insecticide was very toxic to lady beetles whereas Entrust®, an organic insecticide, was not. Results will be discussed in the context of these cropping methods and treatments.

ANTHROPOLOGY AND SOCIOLOGY

One-Child Family Policy, What Do We Obtain? ZHENG WANG* and ELMER GRAY, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

In 1979, the Chinese Government issued the One-Child Family Policy which placed restrictions to control the population size in order to slow down the population growth. Almost three decades have passed since the policy application. During the period, how did the policy impact on the family control and sex ratio become the hottest

topic for many scholars both inside and outside China? The present study, done in the summer of 2008 in Shenyang, China, was conducted to review the past situation, discuss the effect of the policy today, and especially, look into the possible changes and trends in family sizes and sex ratios. The study included 976 university students with equal number of males and females who were surveyed on the number of children in parental, present and projected generations. Average numbers of children were 4.50, 1.64, and 1.73, and secondary sex ratios were 101.22, 108.27, and 107.12 for the parental, present and projected generations, respectively. In the present generation, approximate percentages of families stopping with 1, 2, and 3 children were 54, 34, and 9%, respectively. For desired families in the projected generation, the approximate percentages wanting 0, 1, 2, and 3 children were 9, 19, 66 and 4%; respectively. Comprehensively viewing the history and combining our study results, as well as China's response to the policy, the conclusions include: 1) The One-Child Family Policy did effectively decrease the family size and stabilize the fertility rate at a reasonable level for nearly twenty years from 1990 till today, 2) Extremely strict restrictions and son preference forced people to conceal their true family compositions and to take measures to minimize the number of girls, which directly impacted the sex ratio to be abnormally high, and 3) In the future, it is likely that the policy will be relaxed, permitting two-child families which are preferred by majority of couples. The two-child families will be both genders with male being born first. The sex ratio may gradually recover from male dominance to be more balanced. Also, it is likely that the order of birth may not be a strong consideration for families in the future.

Increasing Public Interest in the Celebration of Chinese New Year in the Bluegrass Region. CHERYL PAN, Department of Curriculum and Instruction, University of Kentucky, Lexington KY 40506.

Chinese New Year is the most important holiday for people of Chinese origin and it is associated with many Chinese cultural traditions. The objective of this project was to assess the public interest in this holiday in the Bluegrass Region. Chinese New Year celebration was held in Lexington for the last three years. The programs consisted of performance of Chinese songs and dances. Data were collected on public attendance, media reports and involvement of public officials. A survey was also conducted on a convenient sample of participants to the events. The number of people attending the public celebration has increased from about 300 in the first year to nearly 1500 in the last year. The racial composition of the participants has changed from mostly Chinese Americans to mostly Caucasians outnumbering Chinese Americans. The supporting public identities for latest event included the school systems, universities, city government and the governor's office. Majority of the participants were people 20–50 years old with some school age children and senior citizens. These results indicate that there is a growing interest in the celebration of Chinese New Year in the Bluegrass Region, as a result of efforts by prominent artists, community organizations and government agencies.

BOTANY

Influence of Elevation, Host Species, and Host Size on the Density of Mistletoe, *Phoradendron robustissimum* (Viscaceae) in Costa Rica. JESSICA R. PRICE, Department of Biology, Berea College, Berea, KY 40404.

Phoradendron robustissimum (Viscaceae) is an evergreen, dioecious, epiphytic hemiparasitic plant dispersed by birds of certain host trees in Mexico, Costa Rica, and other Central American countries. Its haustorial roots tap into the xylem tissue for water and minerals, while the plant produces its own photosynthate. This study was conducted during the spring of 2008 in the Monteverde Region, Puntarenas Province, of Costa Rica, to examine whether the density of mistletoe, *Phoradendron robustissimum*, is a function of the host species, host size (diameter at breast height [dbh]), and/or elevation of the host species. Eighty-eight *Phoradendron robustissimum* infested trees were identified, and station botanists verified those identifications. Data were then collected on the density of mistletoe by visually counting mistletoe clumps of the infested tree species. The dbh was taken on each host species and the elevation was recorded for each location of *Phoradendron robustissimum*. Within the study site, *P. robustissimum* was found on *Sapium glandulosum* (43 host trees), *S. laurifolium* (2 host trees), and *S. macrocarpum* (43 host trees) in the Euphorbiaceae. Both ANCOVA and ANOVA were run but no significance was found in the data for either test. The small data sample of *Sapium laurifolium* was not used in either of these statistical tests.

Roadside Pennycress (*Thlaspi alliaceum*, Brassicaceae) in Kentucky (1982–2008): An Invasive Exotic Plant and Brassicaceae Associates. RALPH L. THOMPSON*, Berea College Herbarium, Department of Biology and KATRINA RIVERS THOMPSON, Department of Child and Family Studies, Berea College, Berea, KY 40404.

Garlic or Roadside Pennycress (*Thlaspi alliaceum* L.) is a naturalized, European, fast-growing annual in the Brassicaceae. Its name is derived from the garlic-like odor of the foliage. It typically inhabits ruderal highway roadsides and other disturbed corridors. In the last three decades, it has become abundant along eastern United States interstates, parkways, and main highways in DE, IN, KY, LA, MD, MO, NC, NJ, OH, PA, TN, VA, and WV. Seeds are dispersed in rural corridors through high traffic volumes, extensive mowing programs, and construction and maintenance projects. Garlic Pennycress was discovered as a new species for Kentucky in 1982 by John W. Thieret. During the 1980s, Roadside Pennycress was reported in six counties, 26 counties in the 1990s, and 40 counties from 2000–2006. In 2007–2008, we docu-

mented it in 76 more counties for the current total of 116/120. It was not found in Ballard, Carlisle, Crittenden, and Hickman Counties. Roadside Pennycress has 78 associated species recorded among the 116 counties. Fifty-nine are naturalized (75.64%) of which 24 are state-listed invasive species (40.68%). Twelve species in the Brassicaceae (11 naturalized, 1 native) are associates: *Cardamine hirsuta, Barbarea vulgaris, Draba verna, Thlaspi perfoliatum, Thlaspi arvense, Brassica rapa, Lepidium campestre, Alliaria petiolata, Capsella bursa-pastoris, Arabidopsis thaliana, Erysimum repandum*, and *Lepidium virginicum. Thlaspi alliaceum* should be strongly considered listing as an invasive exotic pest plant based on its rapid spread into fallow fields, cultivated fields, pastures, and forested borders in Kentucky.

Preliminary Floristic Survey of Old Mulkey Meeting House State Historic Site, Monroe County, Kentucky. RALPH L. THOMPSON*, Berea College Herbarium, Department of Biology, Berea College, Berea, KY 40404 and RONALD L. JONES, Department of Biological Sciences, Eastern Kentucky University, Richmond, KY 40475.

Old Mulkey Meeting House State Historic Site, a 32.0 hectare tract of mostly forested land, is located in Monroe County approximately 0.7 km south of Tompkinsville. It was designated a state historic site in 1931. Old Mulkey was originally built in 1804 during the "the Great Awakening" religious movement with John Mulkey as the first preacher. Old Mulkey has historic significance because it is the oldest meeting house in Kentucky, the oldest wooden building of its kind west of the Appalachians, and cemetery of several Revolutionary War soldiers and early pioneer settlers. The historic site lies in the Eastern Highland Rim of the Interior Low Plateau. Elevation ranges from 232 m at Mill Creek to 274 m on an upland ridge. Bedrock is Mississippian shaly calcareous siltstones of the Fort Payne Formation, and shales, siltstones, and limestones of the Salem and Warsaw Limestone Formation. Residual deep, acidic, and well drained soils are Garmon shaly silt loam, Lowell silt loam, Waynesboro loam, and Waynesboro clay loam. Mixed Mesophytic Forest is found in a spring-fed stream ravine and on lower to upland side slopes. Oak-Hickory Forest predominates on higher upland elevations. The known vascular flora comprises 358 specific and infraspecific taxa in 244 genera from 100 families. Taxonomic distribution is nine Polypodiophyta, four Pinophyta, and 345 Magnoliophyta (Liliopsida 81 and Magnoliopsida 264). Asteraceae (46), Poaceae (41), Fabaceae (23), Cyperaceae (18), and Rosaceae (15) are the largest families. Sixty-six (18.4%) are naturalized taxa including 37 Kentucky invasive species.

GEOGRAPHY

Threatening Change: Distance and Vicinity to 2001–2005 Temporal Land Cover Change for the Calibration Sites of the 2001 Kentucky Landscape Snapshot Project. DEMETRIO P. ZOURARAKIS, Kentucky Division of Geographic Information, Frankfort, KY 40601.

A set of 338 calibration/training sites utilized in the creation of the Kentucky portion of the 2001 National Land Cover Dataset (NLCD01) by the Kentucky Landscape Snapshot project (KLS) was selected. The sites were characterized by their proximity to temporal, significant land cover change experienced during the 2001–2005 period, based on a dataset created as a 2005 update of the Kentucky portion of the NLCD01, produced during the Kentucky Landscape Census (KLC) project. Straight line – or Euclidean distances to the training sites from areas of land cover change sites (LCCs) and summary statistics were calculated. While "straight line" distance metrics to changed (2001–2005) areas indicated that training sites still persist in their basic land cover type, 50% of the sites were at 1.4 km or less from a LCC. When grouped by class, the mean distance from calibration sites to LCCs showed slight variation: Oak/Deciduous Bottomland, Floodplain and Woodland Wetland (Total = 423 LCCs; Average = 2.21 km); Oak-Pine, and Hemlock-Deciduous (Total = 236 LCCs; Average = 2.21 km); and Pine, Red Cedar and Hemlock (Total = 136 LCCs; Average = 1.06 km); and Oak, Yellow Poplar and Mixed Deciduous (Total = 595 LCCs; Average = 1.56 km). This proximity to potentially significant landscape change areas is of concern, despite that some of the sites were located on "protected" lands.

GEOLOGY

Seasonal Changes in a Eutrophic Lake, Wilgreen Lake, Madison County, Kentucky. RICHARD D. STOCKWELL* and WALTER S. BOROWSKI, Department of Geography and Geology, Eastern Kentucky University, Richmond, KY, 40475.

Wilgreen Lake (Madison County, Kentucky) covers ~169 acres, and was formed in 1966 by damming Taylor Fork. The Wilgreen watershed drains residential developments, modified woodlands, cattle pasture, and some industrial/urban areas in the city of Richmond. The lake is deemed "nutrient impaired" by the EPA. Our main objective is to document the seasonal changes in key lake parameters from summer stratification through fall overturn over four months of sampling, August through November, 2008. We collected temperature, oxygen, pH, and conductivity data from 19 stations at depth intervals of one meter using an YSI multi-probe. Concurrent with collecting these framework data, we took water samples, also at one-meter intervals, and measured phosphate (PO_4^-), ammonium (NH_4^+), and nitrate (NO_3^-) concentration. Summer stratification exerts a large control on processes occurring within the lake, as does stream inflow. August and September surface temperatures ranged from 25 to 26.5°C, considerably cooler than in 2006 and 2007. The summer thermocline was located between 6 to 7 m. Oxygen concentrations are highest in surface layer of the lake, become disoxic below 3 to 4 m, and are anoxic only for the bottom several meters at our deepest stations. In

2008, a much smaller volume of the lake was anoxic. We attribute these annual differences to a much cooler and wetter early summer in 2008. Much higher nutrient concentrations occur near the largest stream inputs into the lake, either from watershed runoff or from anthropogenic sources near the lakeshore such as septic systems.

Determination of Suspended Sediment Concentrations, Instantaneous Suspended Sediment Loads, and Potential Sources for Suspended Sediment, Dry Creek and Morgan Fork, Rowan County, Kentucky. SAMUEL WILLIAMS* and STEVEN K. REID, Department of Physical Sciences, Morehead State University, Morehead, KY 40351 and CHRISTINE MCMICHAEL, Institute for Regional Analysis and Public Policy, Morehead State University, Morehead, KY 40351.

The 2007 Kentucky Environmental and Public Protection Cabinet list of impaired streams identifies a segment of Dry Creek from its mouth to 0.5 miles upstream as partially supporting aquatic life due to sedimentation/siltation and organic enrichment (sewage). Urbanization in the Dry Creek watershed is accelerating. This study provides a snapshot of suspended sediment concentrations (SSC) and instantaneous suspended sediment loads in Dry Creek and Morgan Fork (a major tributary) and includes preliminary results of reconnaissance to identify sources of contamination. Suspended sediment sampling used the methods of Edwards and Glysson (1998). Whenever possible, the equal-width-increment (EWI) method and DH-48 sampler were used. Very high or low discharge events required the use of dip or single vertical sampling. Suspended sediment analysis followed procedures of ASTM D3977-97 and Guy (1969). Discharge (Q) was measured using the velocity-area method or neutrally buoyant object method (Rantz et al., 1982) depending on flow conditions. SSC values range from 0 to 341.30 mg/L. Instantaneous suspended sediment loads range from 0 to 510 tons/day. A crude band has begun to emerge on plots of SSC vs. Q. Potential sediment sources include bank erosion and slumping, highway construction, and poor land management practices. Results of this study have led to a new study involving bank-pinning and measurement of channel cross-sections to quantify sediment contributions due to bank instability. In addition, a focused study of the impact of a KY 519 highway construction project has been initiated.

HEALTH SCIENCES

Human Sex Ratio and Family Size for a Selected Sample from the India Population in 2007–2008. ARCHANA LAKKARAJU* and PRAMOD GUPTA, Department of Public Health and ELMER GRAY, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

The human sex ratio is of great interest especially in highly populated countries such as India. When there exists a strong gender preference, efforts to limit population growth may impact the gender balance. In 2007 and 2008, students at nine colleges in Andhra Pradesh, India, were surveyed for family size and secondary sex ratio data. The 1190 respondents (595 of each gender) provided data on parental, present, and projected generations. Average numbers of children were 4.27, 2.99, and 2.10 and sex ratios (males: 100 females) were 101, 87, and 99 for families in the parental, present, and projected generations, respectively. For the present generation, percentages of families stopping with 1, 2, 3 and 4 children were 6.6, 32.9, 32.8 and 17.1%, respectively. A total of 123 or 10.4% of the families had more than four children. For desired families, the percentages wanting 0, 1, 2, 3, and more than 3 children were 5.0, 14.9, 68.6, 8.47, and 7.94%, respectively. More than two-thirds (68.6%) of the respondents desired two children. For desired families of two and three children, preferences were for both sexes with the male being born first. Within all desired families, all males were preferred over all females, indicating a continuing son preference. However, the presence of both sexes in families of the present generation and the preference for both sexes in families of the projected generation were associated with smaller families than those with all male.

Life Expectancy and the Human Sex Ratio. PRAMOD R. GUPTA*, Department of Public Health and ELMER GRAY, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

Both life expectancies and secondary sex ratios vary among countries of the world. The secondary sex ratio (males: 100 females) changes with advancing age of life. The objectives of the present study were to characterize life expectancies and sex ratios for countries of the world and to explore possible relationships between these two indices of the human population. Data for the year 2006 were obtained for 209 countries from Central Intelligence Agency World Fact Book (ISSN 1553-8133). Life expectancy data were available for males and females and sex ratio data were provided for different stages: at birth, under 15, 15 to 65, over 65, and total. Life expectancies ranged from 32.6 in Swaziland (M 32.1 to F 33.2) to 83.5 in Andorra (M 80.6 to F 86.6). Worldwide women live longer than men in virtually all countries; female mortality rates are lower than males at practically all ages. Averages and ranges for the sex ratios (males per female) were: 1.049 (1.0 to 1.17 at birth), 1.041 (0.96 to 1.13 under 15), 1.021 (0.70 to 2.24 for 15 to 65), 0.812 (0.46 to 2.84 over 65), and 1.004 (0.77 to 1.84 total). The sex ratio at birth is highest. Due to generally higher life expectancy of females, sex ratio tends to even out in adult population and result in an excess of females among the elderly. Linear correlations between sex ratios for adjacent stages of life were positive and significant; however, correlations between ratios of separated stages were not significant. Correlations between life expectancy and ratios at birth and under 15 were positive and significant but not significant for other stages or for the total sex ratio because the most important single factor in

increase in life expectancy is reduction of death in childhood. There were great variations in life expectancy worldwide, mostly caused by differences in public health, medicine and nutrition from country to country.

Effects of a Body Conditioning Class on the Body Weight and Body Composition of College Students. JO SLOAN*, CECIL BUTLER, LINGYU HUANG, and CHANGZHENG WANG, Human Nutrition Program, Kentucky State University, Frankfort, KY 40601.

The lack of physical exercise is one of the major factors responsible for the obesity epidemic in the United States. Kentucky State University offers a body conditioning class to college students to motivate them to be active and provide instructions on how to adopt various exercises into their daily life. The objective of this study was to determine the potential impact of this class on body weight and composition of college students. A total of 50 students enrolled in the class met twice per week for 17 weeks. Each class lasted 50 minutes. The students were given instructions first and then engaged in a series of flexibility exercises, track exercises and localized body strengthening activities. The students were measured for body weight, height and body fat percent at the beginning and the end of the semester with a Tanita TBF -521 body composition analyzer. No consistent changes in body weight and fat percent were observed among these students during the semester. This result suggests that modifications of the course content may be necessary to encourage the students to become more active physically.

PHYSIOLOGY AND BIOCHEMISTRY

Sugar Modifies the Water Activity and Water Phase Salt Content of Smoked Paddlefish Meat. KIA RODRIGUEZ*, CECIL BUTLER, LINGYU HUANG, CHANG ZHENG WANG, RICK ONDERS and STEVEN D. MIMS, Human Nutrition Program, Kentucky State University, Frankfort, KY 40601.

The Food and Drug Administration requires that smoked fish contains a minimum of 3.5% water phase salt to ensure the water activity of the product is low enough to inhibit the growth of *C. botulinum*. The objective was to determine the effects of sugar added to the brining solution on the water activity and water phase salt content of smoked whole paddlefish. Three paddlefish each were brined in a 15% salt solution with 92.5 g, 185 g or 370 g of sugar per gallon of brine in a vacuum tumbler for 1 hr. At the end of the brining, fish were rinsed in tap water and left to dry at 4°C overnight. They were hot smoked until the internal temperature reached 145°C for 30 min. After cooling down in a refrigerator, the smoked fish were vacuum-packed and stored at −20°C before analysis. The smoked meat was homogenized in a grinder. Two-gram samples were soaked in distilled water for two hrs. The supernatant was used for salt analysis by a salt analyzer. The water activity of fish brined with 92.5 g sugar was above the safe range. Added sugar tended to reduce the salt content of the smoked fish meat, but kept the water activity below 0.95. The results indicate that adding sugar into the brining solution may help to reduce water activity and avoid extreme saltiness without compromising safety of smoked fish products.

PSYCHOLOGY

The Impact of Enhanced AD/HD Knowledge on Successful Malingering of Childhood Symptoms on the Wender Utah Rating Scale. CASSIE M. WATKINS* and SEAN P. REILLEY, Department of Psychology, Morehead State University, Morehead, KY 40351.

Attention Deficit/Hyperactivity Disorder (AD/HD) is a prevailing disorder among children and increasingly more common among adults. It is possible for college students with a diagnosis of AD/HD to get special accommodations from their college or university such as untimed tests and rescheduling of exams, not to mention the stimulant medications involved. With these potential benefits, students could attempt to feign AD/HD symptoms especially on behavioral ratings scales commonly used to establish the diagnosis. The current study examines the resiliency of the Wender Utah Rating Scale (WURS) to malingered childhood AD/HD symptoms following enhancements of AD/HD knowledge. The WURS is a unique empirical instrument for evaluating adults' retrospective reports of childhood AD/HD. Previous research has shown the WURS to be significantly more effective in preventing falsification of AD/HD compared to other behavioral rating scales. Using an experimental approach, college students were assigned to an AD/HD knowledge enhancement condition or a control condition. Pre-post tests were completed regarding the participants' knowledge of AD/HD and the impact of this knowledge on subsequent instructions to report honestly or to fake AD/HD childhood symptoms on AD/HD rating scales, including the WURS. Our data supported the notion that the WURS is affected by malingering similar to other AD/HD rating scales. The results are discussed in relation to adult AD/HD assessment of childhood symptoms. Research supported by a Morehead State University Undergraduate Research Fellowship and a prior grant from KY EPSCoR.

The Impact of Malingering on the Child and Current Symptoms Scales for AD/HD. HANK SCOTT*, RACHEL COOLEY and SEAN P. REILLEY, Department of Psychology, Morehead State University, Morehead, KY 40351.

Attention deficit hyperactivity disorder (AD/HD) is a commonly diagnosed mental health disorder among children, adolescents, and adults. One widely used measure for assessing AD/HD symptoms is the use of behavior rating scales, such as the Barkley & Murphy Childhood and Current Symptoms Scales. Little research has addressed whether knowledge of AD/HD is necessary to successfully malinger on these particular rating scales. Using a mixed experimental approach, the current study evaluated the impact of reviewing information about AD/

HD vs. a control condition in which non-AD/HD mental health information was provided on subsequent successful malingering of Childhood and Current Symptom Scales scores as well as Social Phobia, a control condition. The present data indicated that even without increased knowledge of AD/HD, it is possible to successfully malinger on the Barkley and Murphy Scales. The results are discussed in terms of enhancing adult AD/HD assessment. Research supported in part by a prior grant from the KY EPSCoR.

The Impact of Studying Specific AD/HD Symptom Information on AD/HD Knowledge and Malingering of AD/HD Symptoms. KELLY D. GRUBER* and SEAN P. REILLEY, Department of Psychology, Morehead State University, Morehead, KY 40351.

Recognition and diagnosis of Attention Deficit/Hyperactivity Disorder (AD/HD) is increasing among mental health providers of adults with the disorder. Adults diagnosed with AD/HD may be eligible for college-level academic accommodations, stimulant medications, or disability. As a result of current technology, information pertaining to diagnostic symptoms, general knowledge, case histories, and treatment of AD/HD is easily accessible. Multiple sources of information via the internet and library disclose a large amount of information about AD/HD. Studying these sources of AD/HD information has been shown to increase physician's knowledge of AD/HD. In malingering research concerning AD/HD, an omitted area concerns the impact of knowledge on successful malingering. Using an experimental approach, the current study tested and found that specific enhancements in diagnostic information regarding AD/HD symptoms did positively impact AD/HD symptom knowledge, but not other aspects of AD/HD knowledge (e.g., treatment). Symptom knowledge provided a unique contribution to successful AD/HD malingering. The results are discussed in relation to adult AD/HD assessment. Research supported by a Morehead State University Undergraduate Research Fellowship and a prior grant from KY EPSCoR.

The Impact of Math Anxiety and Math Self-Efficacy on Math Performance of Students with Remedial and Non-Remedial Math Deficiencies. EVELYNN HUDSON* and SEAN P. REILLEY, Department of Psychology, Morehead State University, Morehead, KY 40351.

Math anxiety, a form of test anxiety, is a problem frequently encountered in college students taking mathematics classes. Math anxiety can have a serious negative effect on math performance. It is believed several factors influence the development and maintenance of math anxiety. These factors include math skill deficiencies, negative prior math experiences, negative attitudes towards math, and low math self-efficacy. Understanding the impact of math anxiety and other related factors is extremely important given the large amounts of students who arrive unprepared for college level mathematics. In the present study, college students completed a packet of questionnaires including the Abbreviated Math Anxiety Scale and the Math Self-Efficacy Scale and then completed in a counterbalanced fashion the Math Calculation, Math Application, and Nonverbal Reasoning tests from the Scholastic Abilities Test for Adults. Consistent with expectations, high math anxiety provided a moderate impact on math performance, but not for the nonverbal reasoning test. Low Math Self-efficacy also provided a moderate impact on math performance, but not for nonverbal reasoning performance. These relationships were also more pronounced in students reporting remedial math deficits. Finally, partial correlations revealed a significant unique contribution for math anxiety to math performance when controlling for math self-efficacy. Research work was funded in part by a prior grant from the Institute for Regional Analysis and Public Policy at Morehead State University.

The Impact of Reviewing Information from AD/HD Case Studies on AD/HD Knowledge and Malingering of AD/HD symptoms. NORA WEYH*, MATT BERRY, and SEAN P. REILLEY, Department of Psychology, Morehead State University, Morehead, KY 40351.

Attention deficit hyperactivity disorder (AD/HD) is a complex, and frequently diagnosed psychological disorder in children, adolescents, and adults. Behavioral rating scales are among the most commonly used measures in AD/HD evaluations in addition to a clinical interview. A gap in the current assessment literature concerns the susceptibility of AD/HD measures to malingering due to review of multiple sources of public information about AD/HD via the internet. Using an experimental approach, the current study tested whether studying published psychiatric case studies about patients with AD/HD would lead to increased AD/HD knowledge, and successful malingering on common AD/HD rating scales. Our data were supportive of these relationships and the results are discussed in relation to malingering concerns in adult AD/HD assessment. Research supported by a prior grant from KY EPSCoR.

ZOOLOGY

The Perception of Phagostimulants is Context Dependent in Larval *Manduca sexta*. YILI GAN*, JORDAN HARRISON, TRICIA STEPHENS and MARC ROWLEY, Department of Biology, Berea College, Berea, KY 40404.

Manduca sexta larvae are good model organisms for studying caterpillar feeding behavior. Previous research has determined specific reference compounds that act as either feeding stimulants or feeding deterrents. The simplest model of a feeding decision system would suggest that these gustatory inputs would be considered in an additive manner. Thus, we hypothesized that increasing the concentration of stimulants in a mixture would result in increased feeding on that mixture. Conversely, increasing the concentration of deterrents

would result in decreased feeding on that mixture. We tested this hypothesis by assaying feeding behavior on mixtures of the feeding stimulants glucose and inositol and the feeding deterrents caffeine and KCl. Our results indicate that rather than this simple additive model, the feeding decision system of larval *M. sexta* is influenced by the overall pattern of inputs such that addition of stimulants to a mixture does not necessarily result in an increase in consumption.

Behavioral Ecology and Translocation of the Endangered Stephens' Kangaroo rat (Dipodomys stephensi). JESSICA R. PRICE, Biology Department, Berea College, Berea, KY, 40404.

Stephens' Kangaroo Rat (*Dipodomys stephensi*), a federally endangered rodent species in the Heteromyidae, is an endemic, nocturnal granivore known from western Riverside County, California. This rodent is endangered because of accelerated habitat loss through degradation and destruction from urbanization and farming. In the summer of 2008, a research study was conducted on a population of the endangered Stephens' Kangaroo Rat at the Metropolitan Water District of Riverside County by translocating individuals from the area under risk for urban development to the protected Lake Skinner Reserve in Riverside County. Data was collected and observations were made for 48 individuals to better understand the social interactions between and among these individuals for a more successful translocation. Individuals were caught in Sherman live traps using millet and oat bait. Data was collected on each individual trapped including weight and reproductive level; fecal samples also were taken. After each kangaroo rat was ear-tagged, observations were made to identify different interactions between individuals and to better understand home ranges. Each individual was fitted with a radio transmitter and held in captivity in cages to monitor their physical condition. Based on the previous observations done, each individual was moved into an acclimation cage at the release site on the Lake Skinner Reserve. To ensure a successful translocation, the release site was monitored, protected, and managed for the Stephens' Kangaroo Rats. After being monitored in the acclimation cages, individuals were released and radio telemetry data was taken to determine the success of the translocations.

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Compiled by Ralph L. Thompson

Guidelines for Contributors to the Journal

All manuscripts and correspondence concerning the Journal should be addressed to the Editor:

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A. Papers should be in 12-point type on white paper 8.5 × 11 inches, with margins at least 1 inch all around. Double-space throughout the paper (i.e., one full line of space between each two lines of text, literature cited, or tabular data). Do not justify right margins. Indent the first line of each paragraph (but not the first line of entries in the Literature Cited).
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E. The first page should include the running head and, centered near the top of the sheet, the paper's title and the name and address of author(s). These should be followed immediately by the abstract and key words. (The first page should look as much as possible like the first page of articles in J-KAS.) The e-mail address of the corresponding author should be at the bottom of the first page as a footnote under a short line, e.g.,
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Introduction, Materials and Methods, Results, Discussion, Summary (optional), Acknowledgments, and Literature Cited.

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B. Do not include any reference unless it has been published or accepted for publication ("in press"; see below).

5. LITERATURE CITED

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C. The first line of each reference should be typed flush left; the remaining lines should be indented five spaces.

D. Examples of common types of references are given below.

JOURNAL ARTICLE

Lacki, M. J. 1994. Metal concentrations in guano from a gray bat summer roost. Transactions of the Kentucky Academy of Science 55: 124–126.

BOOK

Ware, M., and R. W. Tare. 1991. Plains life and love. Pioneer Press, Crete, WY.

PART OF A BOOK

Kohn, J.R. 1993. Pinaceae, Pages 32–50 in J.F. Nadel (ed). Flora of the Black Mountains. University of Northwestern South Dakota Press, Utopia, SD.

WORK IN PRESS

Groves, S. J., I. V. Woodland, and G. H. Tobosa. n.d. Deserts of Trans-Pecos Texas. 2nd ed. Ocotillo Press, Yucca City, TX.

WORLDWIDE WEB SITES

(Listing of web sites in the Literature Cited is not encouraged, but if it is needed, please follow the guide below.)
Smith, A. W. 1999. Title of web site. Web site address. Date accessed (06/12/2005)

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FIGURES (LINE DRAWINGS, MAPS, GRAPHS, PHOTOGRAPHS)

Figures must be camera-ready, glossy, black-and-white prints of high quality or laser prints of presentation quality. These should be designed to use available space effectively: a full page or part of one, or a full column or part of one. Figures should be mounted on heavy white board and covered with a protective sheet of paper; photographs to be grouped as a plate should have no space between them. Dimensions of plates must observe page proportions of the journal. Each illustration in a plate may be numbered as a separate figure or the entire plate may be treated as one figure. Include scale bars where appropriate. Lettering should be large enough to be legible after reduction; use lowercase letters for sections of a figure. Figure captions should be self-explanatory without reference to the text and should be entered together on a page separate from the text. Number figures in Arabic numerals. Statistics presented in figures should be explained in the caption (e.g., means are presented + SE, n = 7). The word "Figure" should be spelled out in the text (Figure 1) and the caption—Figure 1. Description.

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